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ISOLASI, EFEKTIVITAS, DAN KARAKTERISASI BAKTERIOFAG LITIK *Salmonella enterica* SEBAGAI BIOKONTROL PENYAKIT GASTROENTERITIS

ISOLATION, EFFECTIVENESS, AND CHARACTERIZATION OF *Salmonella enterica* LYTIC BACTERIOPHAGE FOR BIOCONTROL OF GASTROENTHERITIS

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ABSTRAK

Salmonella enterica merupakan salah satu bakteri patogen penyebab gastroenteritis yang ditransmisikan melalui air dan makanan terkontaminasi yang sering terjadi pada negara berkembang. Beberapa strain *Salmonella enterica* multi-resisten terhadap berbagai antibiotika. Bakteriofag litik pada famili *Siphoviridae* dapat menjadi solusi alternatif dalam mengurangi kejadian gastroenteritis oleh *Salmonella enterica*. Tujuan penelitian ini adalah untuk mengisolasi, mengetahui kemampuan bakteriofag litik *Lytic Bacteriophage* 1 (LB1) dalam melisis inangnya yaitu *Salmonella enterica* penyebab gastroenteritis, serta mengetahui karakterisasi bakteriofag litik LB 1 sebagai biokontrol penyakit gastroenteritis. Bakteriofag litik diisolasi dari pembuangan limbah domestik menggunakan teknik *double layer plaque*. Bakteriofag litik diidentifikasi berdasarkan morfologi plak, struktur litik, inang, aktivitas lisis sel bakteri *Salmonella enterica*, stabilitas dalam kondisi *buffer* yang berbeda dan karakterisasi protein. Bakteriofag litik LB1 hanya menginfeksi sel *Salmonella enterica.* Hasil pemeriksaan dengan menggunakan *Transmission Electron Microscope* (TEM), bakteriofag litik LB1 termasuk ke dalam famili *Siphoviridae*. Morfologi kepala hexagonal-icosahedral berdiameter 72,7 nm, dengan ekor non-kontraktil berdiameter 17,3 nm dan panjang 100 nm. Bakteriofag litik LB1 memiliki stabilitas terbaik dalam buffer Ringers suhu 4 ^{o}C yang ditunjukkan dengan penurunan plak sebesar 28% setelah 3 minggu penyimpanan. Hasil pengujian efektivitas menunjukkan bahwa bakteriofag litik LB1 dapat mengurangi populasi sel *Salmonella* sebanyak 67,12% setelah 8 jam inkubasi. Bakteriofag litik LB1 memiliki 8 protein yang berbeda dengan berat molekul yang beragam 11,4 kDa; 19,6 kDa; 23 kDa; 33 kDa; 58,3 kDa; 77 kDa; 94.5 kDa; 133 kDa. Studi ini menunjukkan bahwa bakteriofag litik LB1 yang diisolasi pembuangan limbah domestik dapat secara efektif mengurangi *Salmonella enterica* dengan cara melisis sel bakteri. Bakteriofag litik LB1 berpeluang dapat digunakan sebagai biokontrol penyakit gastroenteritis yang disebabkan oleh *Salmonella enterica.* Stabilitas terbaik bakteriofag litik LB1 pada penyimpanan dalam buffer Ringer di suhu dingin (4^{o}C), memiliki karakterisasi famili

Siphoviridae, dapat mengurangi *Salmonella enterica sebanyak* 67.12% setelah 8 jam inkubasi, dan memiliki berat molekul 11,4-133 kDa.
Kata kunci: bakteriofag litik, gastroenteritis, *Salmonella.*

ABSTRACT

Salmonella enterica is one of pathogenic bacteria causing gastroenteritis transmitted by water and food contamination which commonly occur in developing country. Some study reported that *Salmonella* serovar *enterica* strains were multi-resistant to various of antibiotics. Lytic bacteriophage in Siphoviridae family offered a good solution to reduce gastroenterytis disease caused by *Salmonella enterica*. This reseach aim was to isolate, effectivity test of LB 1 and to characterize lytic bacteriophage as biocontrol of gastroenterytis. Methodology and results were LB1 lytic bacteriophage was isolated from domestic waste using double layer plaque technique, was determined by the plaque morphology, the structure, the host range, the activity to lyse bacterial host cells, the stability of phage on different buffer conditions, and the protein characterization. The results showed that LB1 only infects *Salmonella enterica*. Based on Electron Microscope Observation showed that LB1 is grouped into *Siphoviridae*. It has hexagonal-icosahedral head with 72.7 nm in diameter and long-non contractile tail with 100 nm in diameter. LB1 had a good storage stability in Ringers buffer at low temperature (4^0C), with viability of bacteriophage decreased by 28% after 3 weeks of storage. The effectiveness showed that LB1 could reduce *Salmonella enterica* by 67.12% after 8 hours of incubation. LB 1 has different proteins with molecular weights: 11.4 kDa, 19.6 kDa, 23 kDa, 33 kDa, 58.3 kDa, 77 kDa, 94.5 kDa, and 133 kDa. The conclusion was LB1 was isolated from sewage water were identified to reduces *Salmonella enterica* effectively with concentration of $8.2x10^8$ CFU/mL. LB 1 can be used as a biocontrol of gastroenterytis caused by *Salmonella enterica,* LB 1 has the best stability in buffer ringers in cold temperatures (4^oC) and proven as *Siphoviridae* family, reduced *Salmonella enterica* by 67.12% after 8 hours of incubation, and has protein molecule with molecular weight 11.4 to 133 kDa.
Keyword: gastroentritis, lytic bacteriophage, *Salmonella*.

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INTRODUCTION

Salmonella enterica is a bacterium that causes foodborne disease in humans, which is common throughout the world. Salmonellosis is a disease caused by *Salmonella* bacterial infection. Symptoms are characterized by diarrhea, fever, and pain in the abdomen (Mahamuni *et al*., 2017). If eggs are consumed raw or not cooked properly and consumed by humans, it can cause salmonellosis (Seockmo *et al.,* 2016). Diarrhea and gastroenteritis are the first sequence diseases that cause hospitalization in hospitals in Indonesia with the number of cases of outbreaks of diarrhea in 2010 as many as 2,580 with deaths of 77 cases (CFR 2.98%) (Kemenkes RI, 2014).

In case of severe Gastroenteritis, it uses antibiotics. Excessive use of antibiotics in addition to causing dangerous side effects can also risk the emergence of bacterial resistance to antibiotics (Odonkor and Addo, 2011). The previous studies report that some Salmonella isolated from raw chicken meat are resistant to ampicillin and tetracycline (CDC, 2015). Eng *et al*,. (2012) reported that some Salmonella showed resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole. Antibiotic resistance not only exposes pathogenic bacteria, but can also expose bacteria that act as normal flora, so we need a natural biocontrol.

Siphoviridae bacteriophages family is one alternative to control pathogen bacteria which were reported to infect pathogenic bacteria. The previous studies reported that *Siphoviridae* bacteriophage can reduces *Klebsiella pneumonia* cells causing outbreaks of nosocomial infections because of its ability to caused multidrugs-resistant (Jamal *et al.* 2015). It can reduces *Salmonella* serovar *enterica* isolated from fecal samples (Phothaowrn *et al*. 2019). Lytic bacteriophage are natural and non-toxic methods to reduce and control the growth of human pathogenic bacteria because bacteriophages are part of the gastrointestinal and environmental ecosystems (Bhardwaj *et al.* 2015). The presence of bacteriophages widespread in nature. Occupied environment by host bacteria is a source of existence various types of phage that can be isolated for variety aim (Shende *et al.* 2017). Therapy using lytic bacteriophages is more beneficial because the use is more profitable than antibiotics, bacteriophages only infect the target pathogen, so that the microflora normal in the intestine is not disturbed, second bacteriophages self-replicating in bacteria and completely destroys host bacterial cells through the lysis process kills host bacteria (Strydom and Witthuhn, 2015; Cheng *et al.*, 2018; Harada *et al.,* 2018), and specificity in attacking host target (Kittler *et al.,* 2017; Harada *et al.*, 2018; Santos *et al.,* 2018).

Many studies have reported on the use of bacteriophages as a substitute for antibiotics to combat the growth of pathogenic bacteria, for example The Identification of Salmonella sp contamination and Isolation of Bakteriophage as Biocontrol in Handling Post Vannamei Shrimp Harvesting (*Litopennaus vannamei*) (Anjung, 2016). Saefunida *et al.* (2016) reported that lytic bacteriophage isolates were able to lyse *E. coli* by 92.1%%. Lytic bacteriophages have optimal growth in order to be able to lyse host cells, so that a suitable environment is needed so that lytic bacteriophages can infect host cells and replicate well (Pelzek, 2013). The existence of environmental factors such as temperature and buffer is thought to influence the damage to the structure of elements such as the head, tail, protein, and changes in the structure of DNA so that it affects the production of lytic bacteriophages. Research on lytic bacteriophages is still rarely done in Indonesia, so this research needs to be done to isolate, effectivity test, and characterize of lytic bacteriophage which can lyse *Salmonella* cells causing gastoenteritis.

METHOD

The research was experimental laboratoric by using *post test control group design*. The population was *Salmonella enterica.* isolated from faecal of diarrhea patients in Bogor tested by IMViC test. The samples was sewage water taken from three different places.

Research Instruments

Nutrient Agar (Difco, Becton Dickinson and Company, USA), Nutrien Broth (Difco, Becton Dickinson and Company, USA), Salmonella Shigella Agar (SSA) (Oxoid CM009), Eosin Methylene Blue Agar (Oxoid, Basingstoke, UK), buffer ringers [8 g NaCl, 0.42 g KCL, 0.24 g $CaCl_2$, 0.20 g $NaHCO_3$ in 1 litre H_2O], buffer SM [5.8 g NaCl, 2 g $MgSO_4 \cdot 7H_2O$, 50 mL Tris-Cl (pH 7.5), 5 mL gelatine in 1 litre H_2O], pre-stained protein (Pageruler Prestained Protein Ladder, Thermo Scientific Fermentas Technology, UK), TEM (JEOL JEM-1010, Tokyo, Japan). The isolates used to determine the host range consisted of *Escherichia coli*,, *Proteus mirabilis, Bacillus pumilus*, and *Photobacterium damselae* obtained from the Bogor Culture Collection Agricultural Institute (IPBCC), Department of Biology, Bogor Institute of Agriculture.

Research Flow

1. Isolation of Lytic Bacteriophage

Sampling. The sample used was domestic waste from sewer water and septic tanks. Samples are taken and put in sterile bottles of 5-10 mL. Then the sample is homogenised and then filtration is carried out.

Filtration. A total of 1 mL of domestic waste sample was diluted into 9 mL NB media, then centrifuged at 3,000 rpm for 20 minutes, and filtered using 0.45 µm millipore membrane. The filtrate from the filter was 4.5 mL then mixed with 0.5 mL of *Salmonella* sp. OD600 culture = 1 (10^8 CFU/mL) and 5 ml of Nutrient Broth (NB) was added. The mixture is incubated for 24-48 hours in a Water Bath shaker at 37ºC. The culture was then centrifuged at 3000 rpm at 4°C for 15 minutes. Supernatant was taken with syringe and filtered using 0.22 µm millipore membrane. The filtered supernatant is inserted into a sterile tube.

Double layer Plaque Technique. A total of 100 µL of *Salmonella* sp. lytic bacteriophage stock was diluted into buffers with serial dilutions of 10^{-1} to 10^{-6}. Each was taken as much as 100 µL and each was mixed with 100 µL of *Salmonella* bacteria into a sterile effendorp tube and incubated at 37ºC for 30 minutes. The mixture was added with 7 mL soft agar which had a temperature of 47ºC, poured on NA media. Incubation was carried out at 37ºC for 24 hours and then plaque was formed (Jatmiko, dkk., 2018).

Bacteriophage purification. The plaque that is formed is taken using a pasteur pipette and enrichment is carried out so that the resulting plaque will increase. The plaque was transferred into 10 mL *Salmonella* sp. culture and incubated for 24 hours centrifuged at 3000 rpm at 4°C for 20 minutes, the bacteriophage filtrate was then filtered using a 0.22 µm millipore membrane. The results of the filter in the form of bacteriophage filtrate then carried out on petri dish. The resulting plaque formed is then taken and put into the Ringers buffer. Suspension of bacteriophage were vortex and left for 5-10 minutes at room temperature, then centrifuged at a speed of 3000 rpm, a temperature of 4ºC for 20 minutes for 2 replications. The supernatant was filtered using 0.22 µm millipore filter membrane and then stored as bacteriophage stock (Merabishvili *et al*., 2009).

2. Production of Lytic Bacteriophage

A total of 10 mL of *Salmonella* sp. bacterial culture on NB media was centrifuged at 3000 rpm, at 4^{o}C for 20 minutes. The pellets formed were each infected with 100 µL of lytic bacteriophage. The mixture was incubated at 37^{o}C for 30 minutes, then added 10 mL of NB media and incubated for 24 hours at 37^{o}C. Then the culture was centrifuged at a speed of 3000 rpm, temperature 4^{o}C for 20 minutes. The supernatant formed was taken with a syringe and filtered with a 0.22 µm membrane filter. Each filtered supernatant is put into a sterile tube and stored (Kropinski & Lavigne, 2009).

3. Lytic Bacteriophage Quantification

Phage quantification is measured by calculating the number of plaque formed (Plague Forming unit/PFU per mL). Lytic bacteriophage stock was diluted up to 10^{-8}, then from each dilution of the bacteriophage isolate, 100 µL was added with 100 µL of *Salmonella* sp. bacterial culture that had been incubated for 24 hours on NB media. The suspension was incubated for 30 minutes at 37^{o}C. A total of 7 mL of soft so that the temperature 47^{o}C was mixed, then each was poured into the NA media, incubated at 37^{o}C for 24 hours. Then the formation of plaque (clear zone) is observed and the amount is calculated (Kropinsky & Lavigne, 2009).

4. Efectiveness of Cell Lysis by Lytic Bacteriophage

The testing was by infecting 3.4 x 10^6 PFU/mL lytic bacteriophages with 3 x 10^5 CFU/mL Salmonella bacteria in NB media. Control is done by culturing *Salmonella* sp. bacteria into NB media without the addition of lytic bacteriophages. Each treatment and control were incubated at 0 hour, 2 hour, 4 hour, 6 hour, and 8 hour intervals, by *Total Plate Count* (TPC) using NA to count the number of live bacteria at each time interval.

5. Effect of Buffer and Storage Temperature on The Stability of *Salmonella* Lytic Bacteriophages

Salmonella lytic bacteriophage plaques are purified by removing plaque formed using a pasteur pipette. The plaque is then mixed with the Ringers buffer and the Saline Magnesium (SM) buffer. For control, Nutrient Broth (NB) is used. Each bacteriophage suspension was vortex and left for 5-10 minutes at room temperature, centrifuged at 3000 rpm at 4^{o}C, filtered using a 0.22 µm millipore membrane (Phumkhachorn & Rattanachaikunsopon 2010). Then Double Layer Plaque Technique was performed to determine the concentration of lytic bacteriophages. Each bacteriophage filtrate was stored at room temperature (25^{o}C) and cold temperature (4^{o}C), the Double Layer Plaque Technique was assay on the third, sixth, and nineth, and counted the number of plaques formed in *Salmonella Shigella* Agar (SSA) (Phumkhachorn & Rattanachaikunsopon, 2010).

6. Determination of Specificity of *Salmonella* Lytic Bacteriophage

Each of 100 µL of *Salmonella, Bacillus pumilus, Photobacterim damselae*, and *Proteus mirabilis* cultures that had been grown in NB media in the exponential phase were each mixed with 100 µL of *Salmonella* lytic bacteriophage stock. Then each incubated at 37^{o}C for 30 minute. Each bacterium that had been mixed with Salmonella lytic bacteriophage was performed with a double layer plaque technique. Incubated at 37^{o}C for 24 hours and see the formed plaque (Phumkhachorn & Rattanachaikunsopon, 2010).

7. Morphological Observation of Lytic Phages by *Transmission Electron Microscope* (TEM)

Lysed bacteria in Ringers buffer were dropped 5 µL on the grid (400 mesh) using a micropipette, waited for 20 seconds, then dried with filter paper. A total of 5 µL of acetyl acetate 2% was dropped onto the grid and waited for 1 minute. The grid is dried using filter paper and left for 20 minutes to dry completely. Electron Microscope grids are placed in the holder, left to dry. After the dry specimen is examined using a transmission electron microscope (JEOL JEM-1010) with 10000x-100000x magnification (Carey *et al*., 2006).

8. Molecular Weight Analysis of *Salmonella* Lytic Bacteriophage Protein

Sodium Dodesyl Sulphate-Poly Acrilamide Gel Electrophoresis (SDS-PAGE) was used to determine the molecular weight of lytic bacteriophage proteins analyzed. The marker was the PageRegularTMPrestained Protein Ladder with molecular weights of 10, 17, 28, 34, 48, 55, 72, 95, 130, and 180 kDa respectively. A gel separation of 12% polyacrylamide is placed at the bottom. The concentration of the collecting gel was 7.5% polyacrylamide which was placed at the top after the separating gel was solid. The phytic phages and markers are each mixed with a sample buffer at a ratio of 4: 1 (4 parts of sample and 1 part of sample buffer). The mixture was centrifuged at a speed of 1000 rpm, room temperature, for 20 minutes and heated in boiling water for 5 minutes, put into a gel well with a volume of 45µL. Electrophoresis is run with a current of 20 mA and a voltage of 50 volts for 3.5 hours. Electrophoresis is terminated when the dye sample reaches the 0.5 cm to 1 cm limit from the bottom of the gel. After the electrophoresis ends, the gel is removed from the glass slab and done with silver stain staining (Bradford, 1976).

RESULT

1. Isolation of Lytic Bacteriophage

Isolation was carried out using domestic waste samples consisting of sewage water and septic tank water on Natar, Lampung Selatan.

Tabel I. Isolation of Lytic Bakteriophage on *Salmonella*

Jenis LCRT	A number of Sampel	Bacteriophage Isolation	Bacteriophage Type	Plaque Morphology	Plaque Diameter (mm)
Sewer water 1	2	+	LB1	clear	0,8
Sewer water 2	2	-	-	-	-
Septic tank water	2	-	-	-	-

Bacteriophages can only reproduce at the appropriate host. The presence of clear zones or plaques in Salmonella culture grown in petri dishes is an important parameter of the presence of bacteriophages in the lytic cycle. Bacterioag isolation results obtained 1 bacteriophage isolate and named LB1 (Figure 1).

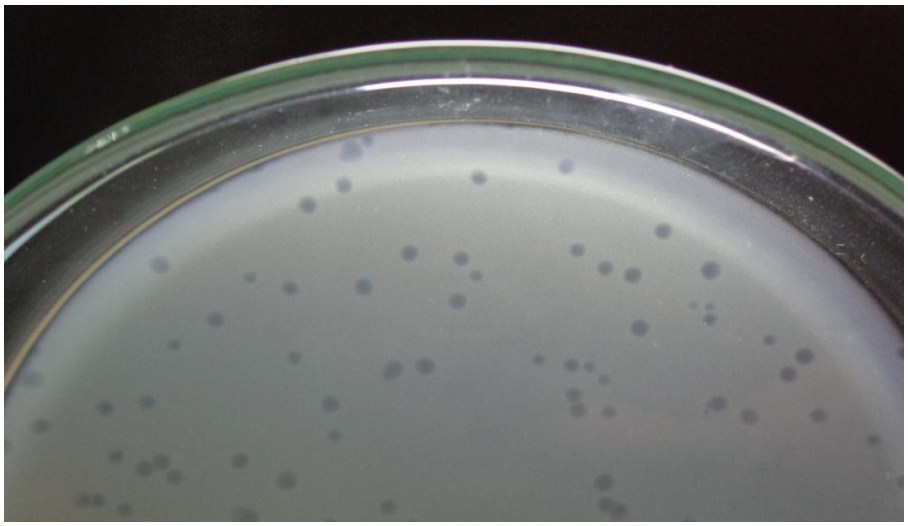


Figure 1. Pattern of LB1 bacteriophage plaque on *Salmonella*

2. Quantification of Lytic Bacteriophage

Purified lytic bacteria LB1 then the amount of concentration was calculated based on the amount of plaque formed. The amount of plaque formed is then calculated in units of Plaque Forming Unit (PFU/mL) (Table 2) which is a measure of the amount of infective fluid volume of the virus. In addition, the calculation of lytic bacteriophage concentration was calculated. LB1 is a lytic bacteriophage with a titer of 8.6 x 10^8PFU / mL, showed that LB1 lytic bacteriophages are effective in infecting *Salmonella*.

3. Lytic Bacteriophage Lysis Effectiveness

The effectiveness of *Salmonella* cell lysis by LB1 lytic bacteriophages carried out by counting bacterial cells directly, the number of bacteria is calculated using *Standar Plate Count* (SPC) (Figure 2).

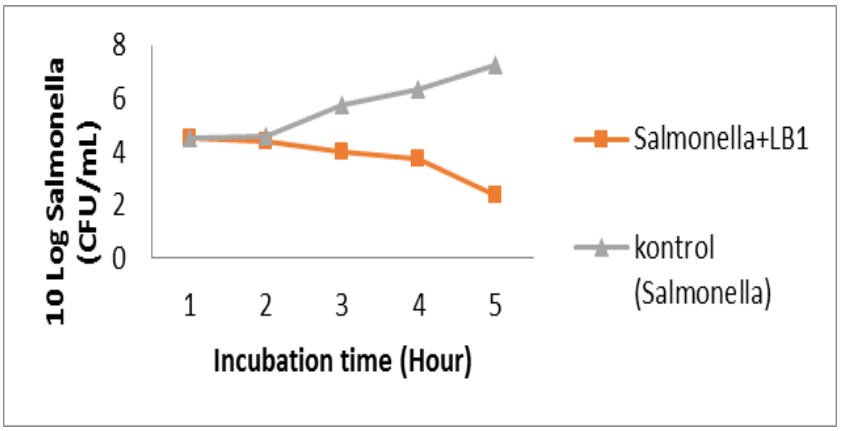


Figure 2. The effectiveness of *Salmonella* sp. cell lysis by bacteriophages. *Salmonella* sp. lysis by LB1 bacteriophage infected (■), and control *Salmonella* sp. without bacteriophage infected (▲).

4. Host Specificity

Determination of the range of lytic bacteriophage hosts is carried out to see the host specificity of the bacteriophages obtained. In determining the range of hosts, LB1 bacteriophages were tested against several other bacteria besides *Salmonella* sp., namely; *Esherichia coli*, *Proteus mirabillis*, *Bacillus pumilus*, and *Photobacterium damselae* (figure 4). Host range test results indicate that LB1 lytic bacteriophages have a narrow range of hosts or are host specific.

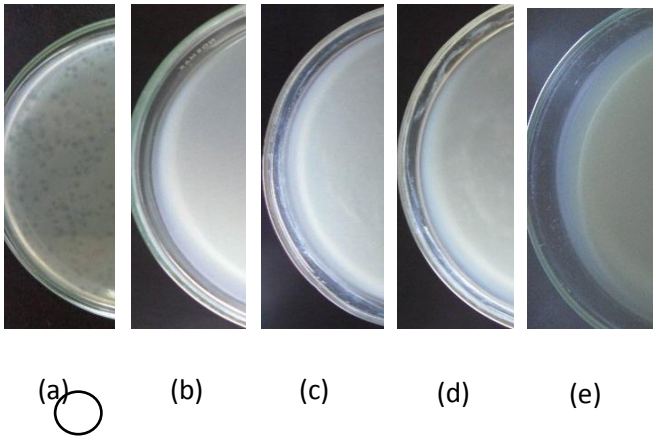


Figure 3. Plaque appears on bacteriophages that are infected with the *Salmonella* sp. (a), whereas bacteriophages which infected by *E. coli* (b), *Proteus mirabilis* (c), *Bacilus pumilus* (d), and *Photobacterium damselae* (e) did not showed plaque appearance. (O) showed lytic bacteriophage.

5. Protein Characterization of Lytic Bacteriophage

Protein levels in LB1 lytic bacteriophages are seen in Figure 4. However, the protein levels in LB1 lytic bacteriophages are relatively small compared to levels of lytic bacteriophage protein in general (protein concentration 158 µg/mL). SDS-PAGE results which were given silver nitrate showed that LB1 lytic bacteriophage suspension contained protein with molecular weights of 11.4 kDa, 19.6 kDa, 23 kDa, 33 kDa, 58.3 kDa, 77 kDa, 94 kDa, and 133 kDa respectively. Protein band formed on LB1 lytic bacteriophage can indicate the LB1 bacteriophage lytic protein.

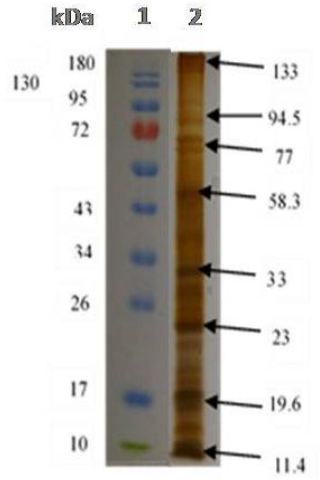


Figure 4. Lytic bacteriophage protein profile with SDS-PAGE; marker (1), and LB1 lytic bacteriophage (2).

6. Morphological Observation Results of Lytic Phages with *Transmission Electron Microscope* (TEM)

Morphological analysis of lytic bacteriophages was performed using the Transmission Electron Microscope JEOL JEM-1010 with a negative staining of uranyl acetate 2%. Lytic bacteriophage observations were carried out using a magnification of 60000x (Figure 5). Lytic bacteriophage LB1 has a hexagonal hexagonal head with a diameter of 72.7 nm, has no contractile sheath, a tail length of 100 nm and a diameter of 18.2 nm. LB1 bacteria are included in the *Siphoviridae* family.

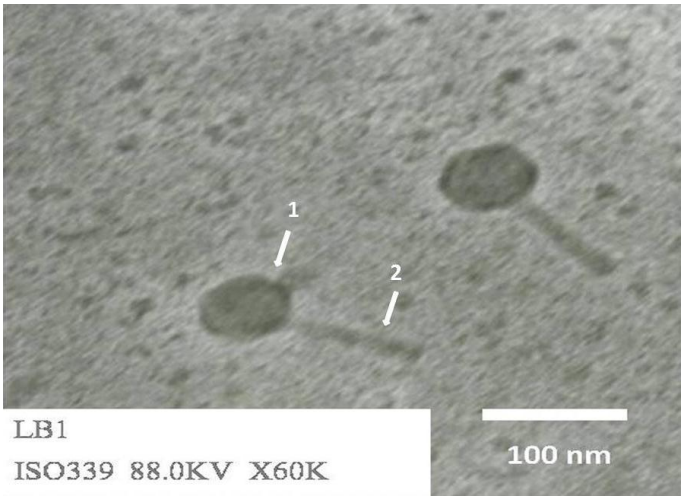
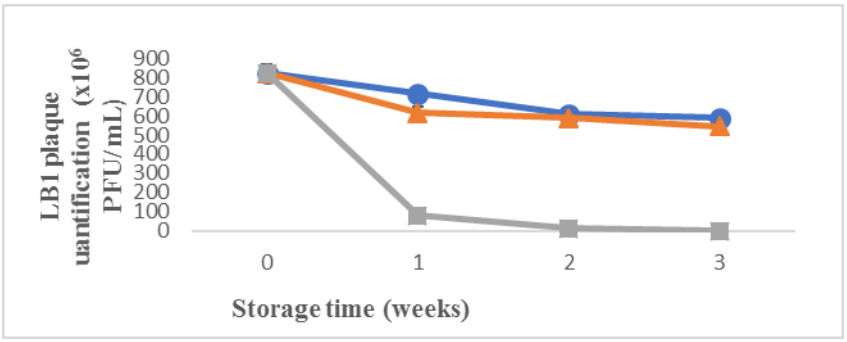


Figure 5. LB 1 lytic bacteriophage by magnification of 60000x; head (1), tail (2).

7. Effect of buffer and storage temperature on the stability of LB1 lytic bacteriophage

The LB1 lytic bacteriophage stability test is carried out by storing bacteriophages in the buffer which aims to find out the best buffer for LB1 lytic bacteriophage storage. Bacteriophage storage treatment uses 2 types of buffer, namely: buffer Ringers and Saline Magnesium (SM) buffer with two different storage temperatures, namely: room temperature (27°C) and cold temperature (4°C). Bacteriophage storage in NB is used as a control. The best bacteriophage LB1 stability is found in bacteriophages stored in Ringers buffer at cold temperature (4°C) (Figure 6).

(a)



(b)

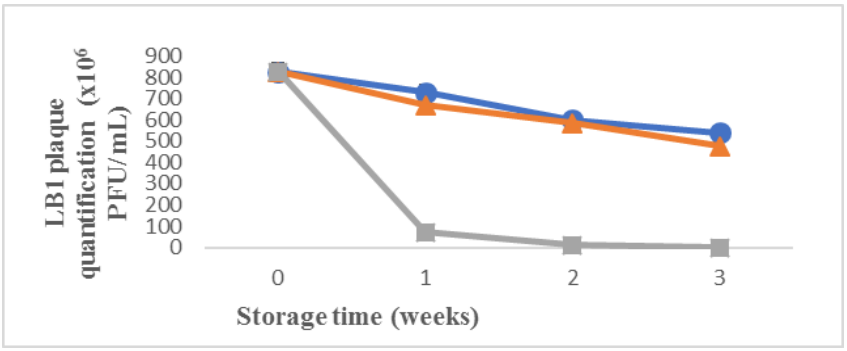


Figure 6. LB1 lytic bacteriophage stability in cold temperature 4°C (a), and room temperature 25°C (b). Each incubated in *Ringers* buffer (●), SM buffer (▲), and NB as control (■).

LB1 lytic bacteriophage showed the best stability in storing ringers buffer at cold temperatures (4°C), this can be seen from the reduction in plaque amount by 28% after 3 weeks of storage, whereas at room temperature (27°C) plaque decreased by 33.9% after 3 weeks of storage. LB1 lytic bacteriophages stored in the SM buffer at 4°C and 27°C experienced greater plaque reduction, namely: 33.3% and 42.4% after 3 weeks of storage. Lytic bacteria LB1 stored in NB medium as a control at 4°C and 27°C did not have plaque at the third week of storage.

DISCUSSION

Phage has a specific bacterial target so that there are no adverse effects on bacteria as microflora, does not cause side effects in humans, storage is relatively stable in different environmental conditions so that production costs are cheap (Hagens and Loessner, 2010). Plaque quantification showed that LB1 has a titer of 8.6 x 10^8 CFU/mL. LB1 bacteriophage was

able to infect *Salmonella enterica* well at 8 hours incubation time. The more bacteriophages produced, the more bacterial cells are lysed by bacteriophages. This allows the opportunity for LB1 to be applied in biocontrol of water and food poisoning. Compared to Jatmiko (2018) hihgest density bacteriophage was S2-St (1,20 x 10^{10} PFU/mL) but the best reduce of Salmonella typhi was B2-St with significant value at the 4th hour, wich showed that B2-St was the best fage even though it has lower density, thought to be due to the type of virus which infects differently, so it has the different ability to infect, replicate and lyses the bacteria, although the bacteriophage cannot lyse bacteria in a manner whole. Plaque size is also influenced by several factors, such as agar concentration, incubation conditions, and the log phase of host bacteria (Kropinski and Lavigne, 2009).

The appearance of plaque indicates that LB1 bacteriophages most of the phages only infect one serotype in one bacterial species. The results of the study of Strydom and Witthuhn (2015), Cheng *et al.,* (2018) and Harada *et al.,* (2018) showed bacteriophages only infect the target pathogen, and specifically attacking host target (Kittler *et al.,* 2017; Harada *et al.,* 2018; Santos *et al.,* 2018). The LB1 host specificity suggests that the surface of Salmonella bacterial cells has receptors that are specific to LB1 phytic phlegm that are not possessed by other bacteria. Phage attaches to cells that are sensitive to stimulation at specific locations in the bacterial cell wall bakteri (Rakhuba *et al*., 2010). Study of Jatmiko (2018) reported that B2St, B3St, S1St, S2St, SL1St, SL3St could lyse *Salmonella typhimurium* and *E. coli* with the formed of plaque, this likely related to similarity in host receptor molecules (Silva *et al.,* 2016) and Deshanda *et al.,* (2018) showed F1, F2, H1, H3, E1, E2 fag could lyse Salmonella, *E. coli* and *Staphylococcus aureus*, while fag F2 lyse Salmonella and *Staphylococcus aureus* only. It means 6 from 7 fag types had larger host range. LB1 bacteria are included in the *Siphoviridae* family, compared to Hardanti *et al.,* (2018) KAS phages have morphological characteristics of *Podoviridae* family with cubic symmetry shaped head and unclear short tail. The effectiveness of lytic bacteriophages in bacterial lysis test aims to determine the exact time needed by bacteriophages to control the proliferation of host bacteria.

The effectiveness of bacteriophage test is done because Salmonella is a common bacterium that causes diarrheal disease after EPEC, so the effectiveness of lytic bacteriophages is expected to be able to control the growth of Salmonella. Several physical and chemical factors such as temperature and ion determine the resistance of bacteriophages. Improper storage will cause damage to the structure of the head, tail, and changes in the structure of DNA (Jonczyk *et al.,* 2012). The stability of LB1 lytic bacteriophages is best in bacteriophages stored in Ringers buffer at cold temperatures (4^oC), this can be seen from the reduction in plaque amount by 28.% after storage for 3 weeks The reduction in the amount of bacteriophage plaque stored in the Ringers buffer at cold temperatures (4°C) has a relatively smaller reduction compared to the storage of bacteriophages in the SM buffer. This is because the presence of Ca^{2+} and Mg^{2+} ions contained in the Ringers buffer can simultaneously increase the number of bacteriophage plaques caused by these multivalent ions can increase the efficiency of phage adsorption into host cells (Cele, 2009).

Capsic and tail of lytic bacteriophage consists of protein. The constituent proteins vary and have different functions. These functions include the protection of bacteriophages against their resistance in the environment and play a role in the process of replication to cause lysis of host cells. The protein possessed by LB1 lytic bacteriophages tends to be small in every milliliter. LB1 has a protein concentration of 158 μg/mL. Through SDS-PAGE we can find

variations of protein molecular weight in lytic bacteriophages. The variation in protein molecular weight indicates the proteins that make up lytic bacteriophages.

CONCLUSSION

LB1 was isolated from sewage water were identified to reduces *Salmonella enterica* effectively with concentration of $8.2x10^8$ CFU/mL. LB 1 can be used as a biocontrol of gastroenterytis caused by *Salmonella enterica*, LB 1 has the best stability in buffer ringers in cold temperatures (4^oC) and proven as *Siphoviridae* family, reduced *Salmonella enterica* by 67.12% after 8 hours of incubation, and has protein molecules with molecular weight 11.4 to 133 kDa.

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