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# Biological characteristics and lytic effectiveness of phages isolated from domestic wastewater against indigenous *Salmonella* spp.

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## Abstract

**Aim :** This study was carried out to isolate and study the effectiveness of lytic phage from domestic wastewater to reduce the population of *Salmonella* spp. in patients suffering from diarrhea and to characterize biological phages.

**Methodology :** The lytic phages from several domestic wastewater were identified using a transmission electron microscope to know morphological phages. After identifying the molecular weight protein by sodium dodecyl sulphate-polyacrylamide gel electrophoresis, to know the effectiveness, the lytic phages were infected to *Salmonella* spp. from diarrheal disease patients and non-pathogenic *Escherichia coli*. Phage stability on thermal, pH, and buffer was then analyzed to determine the biological characteristics.


**Results :** Three lytic phages (F-SB1, F-SB2, and F-SB3), successfully isolated from domestic wastewater, showed an icosahedral head with a short or long tail as their morphological characteristic. These phages were morphologically similar to the phages of family Siphoviridae, Myoviridae and Podoviridae. The three isolated lytic phages were stable at 27 °C to 37 °C, pH 4-7 in sodium magnesium buffer and effectively decreased the population of *Salmonella* spp., however could not lyse *E. coli*.

**Interpretation :** All the isolated lytic phages in this study can contribute as cocktail phages in decreasing the population *Salmonella* spp.

**Key words:** Antibiotic resistant, Domestic wastewater, Lytic phages, Salmonellosis, *Salmonella* spp.

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## Introduction

*Salmonella* spp. is a Gram-negative rod-shaped bacterium that belongs to family Enterobacteriaceae. *Salmonella* spp. can be found in several environments, including gills and intestines of animal (Toroglu *et al.,* 2009). All the strain of *Salmonella* spp. have been reported as important pathogens in human and animals (Budiarti *et al.,* 1991; Hoelzer *et al.,* 2011; Kuhn *et al.,* 2012; Eng *et al.,* 2015; Bertelloni *et al.,* 2016). *Salmonella* spp. are medically important foodborne pathogens, and one of the major cause of human and animal diseases. *Salmonella* species can cause foodborne diseases in humans. In Japan, there were 1505 cases of *S. oranienburg* infection in children due to contamination from snack semi-dry cuttlefish (Miyakawa *et al.,* 2006). Ao *et al.* (2015) reported that *Salmonella* spp. is a key global cause of salmonellosis, which causes non-typhoidal diseases, such as diarrhea. There are 93.8 million reported cases of gastroenteritis around the world due to *Salmonella* species, causing 155,000 diarrheal deaths each year (Majowicz *et al.,* 2010).

Many *Salmonella* spp. have been reported to be resistant to various antibiotics or antimicrobial agents. In Indonesia, they are resistant to antibiotics, including ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, cephalothin, ceftriaxone, norfloxacin, ciprofloxacin, erythromycin and streptomycin (Tjanadi *et al.,* 2003; Kusumaningrum *et al.,* 2012). In other countries, it has been also reported that *Salmonella* spp. are resistant to ceftiofur, gentamicin, nalidixic acid, enrofloxacin, amoxicillin and nitrofurantoin (Matias *et al.,* 2015; Ejo *et al.,* 2016). Alternative approaches to control the emergence of antibiotic resistance in bacteria are urgently needed. It has been reported that phage cocktails are effective in reducing the population of EPEC K1.1 (Arivo *et al.,* 2016), *Listeria monocytogenes* (Guenther *et al.,* 2009), *Enterobacter sakazaki* (Zuber *et al.,* 2008), *Salmonella* sp. (Turki *et al.,* 2012) and *S. enteritidis* (Hungaro *et al.,* 2013).

Several phages have been found in river water (Kusmiatun *et al.,* 2015), domestic wastewater (Arivo *et al.,* 2016), wastewater from local dairy farm (Nugroho *et al.,* 2016), wastewater of poultry processing industries (Quiroz *et al.,* 2016), marine and terrestrial environments (Doss *et al.,* 2017) that can lyse pathogenic bacteria. It can be used against antibiotic resistant bacterial fish pathogen (Prasad *et al.,* 2011; Dinkar *et al.,* 2017). The potential of using lytic phages in nanotechnology and nanomedicine is expected to solve antibiotic resistance issues. In recent years, application of phages as a nanotechnology to control bacterial pathogens has received new interest. Phages are safe, natural and highly targeted antibacterial agents that specifically kill bacteria and can be targeted to kill human pathogens (Sartika *et al.,* 2012). As phages have different morphology, there is an interest in characterizing the morphology and studying the effectiveness of phages to control *Salmonella* population. It is also important to study the biological characteristics of the phages because environmental factors such as pH, temperature and buffer may destroy the elemental structures such as head, tail, protein, and change the DNA structure which can affect the production of phages.

Wastewater, which is commonly contaminated by *Salmonella* spp., is a frequent source of food contamination. The morphological and biological properties of large group of phages isolated from urban sewage have been reported by Kurek *et al.* (2016). However, there is no information about biological characteristics and effectiveness of indigenous lytic phages isolated from domestic wastewater to lyse *Salmonella* spp. present in patients suffering from diarrhea. In view of the above, this study was conducted to isolate the lytic phages of indigenous *Salmonella* spp. and their ability to reduce the population of *Salmonella* spp.

## Materials and Methods

**Isolation and visualization of lytic phages:** Domestic wastewater were of collected from seven different locations in Dramaga, Bogor, Indonesia were used as sources of phage isolates. Three bacterial isolates of *Salmonella* spp. (S1, S2 and S3), used as hosts in this research, were isolated from 200 samples of diarrhea patients in the Laboratory of Animal Biotechnology and Biomedical, Research Center of Biological Resources, Bogor Agricultural University. Approximately, 1 ml of domestic wastewater sample was diluted in 9 ml of nutrient broth medium and centrifuged at 4000 x g for 30 min. The supernatant was filtered using filter membranes Ø= 0.45 µm (Sartorius, Gottingen, Germany). Approximately, 4.5 ml of filtrate was mixed with 0.5 ml of *Salmonella* culture. ($10^8$ cfu $ml^{-1}$). The suspensions were incubated in a waterbath shaker (Certomat WR) for 24 hrs at 37 °C. The culture was centrifuged at 2800 x g for 20 min at 4 °C. Approximately, 3 ml of the supernatant was filtered using a filter membrane (Ø= 0.22 µm). The plaque assay was selected by the double layer agar method. About 100 µl of filtrate was added to 100 µl of *Salmonella* culture and incubated at 37 °C for 30 min. The suspensions were added to 5 ml soft agar (47 °C) and poured into nutrient agar medium. The plates were incubated at 37 °C for 24 hrs.

The phages were quantified by double layer method and measured by counting the number of plaque forming units (pfu $ml^{-1}$). A transmission electron microscope was used to observe the morphology of the phages. Approximately, 10 µl of stock solution of phage was dropped onto the grid for 30 sec and then dried with filter paper. About 5µl of 2% uranyl acetate solution was also dropped onto the grid for 1 min and then dried with filter paper for 60 min. The dried specimens were placed on the holder and were observed with TEM JEOL JEM-1010 at the Eijkman Institute for Molecular Biology, operated at 80 kV at a magnification of 80000 x - 100000 x.

**Identification of phage proteins:** Phage protein analysis was carried out by mixing a stock of sample buffer (2 ml mercaptoethanol, 4 ml glycerol, 0.3 g Tris, 2 ml bromophenol blue 0.1% at pH 6.8) and 0.92 g of sodium dodecyl sulfate with stock

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phage. The molecular weights of the phage proteins were analyzed by sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) with 12% acrylamide (Laemmli, 1970).

**Effectiveness of *Salmonella* spp. lytic phages:** Lytic phage suspensions were tested in *Salmonella* S1, *Salmonella* S2, *Salmonella* S3 and non-pathogenic *E. coli.* The test was conducted using a double layer agar technique. Approximately, 100 ml of *Salmonella* spp. culture ($10^8$ cfu $ml^{-1}$) grown in nutrient broth medium was centrifuged at 2800 x g at 4 °C for 30 min with three replications. Both controls (pellet without phage) and treatments (pellet with phage $10^4$ pfu $ml^{-1}$ and 3 x $10^4$ pfu $ml^{-1}$) were incubated at 37 °C for 30 min. Each treatment was added to 50 ml of nutrient broth medium and incubated at 37 °C. The $OD_{600nm}$ value of each culture was determined every hour.

**Stability analysis of lytic phages:** Stability of lytic phage was analyzed at different temperature (27°C, 37°C, 45°C, 55°C and 60°C) and pH (4, 5, 7, 9 and 11). Nutrient broth medium was used as a control. The purified plaque was mixed with 3 ml of 25% Ringer buffer, 3 ml of sodium magnesium buffer and 3 ml of phosphate buffer saline and then incubated for 10 min at room temperature. The suspensions were then centrifuged at 4°C and filtered through 0.45µl filter membrane. The centrifugation containing supernatant of phage ($10^6$ pfu $ml^{-1}$) was taken and re-filtered using a 0.22 µl filter membrane. The stability of phages to temperature, pH and buffers were determined by platting it using double layer agar technique.

**Statistical analysis:** All data in this study were determined by the means value of replicates in each analysis. The means of replicates in analysis of effectiveness of lytic phages, stability of lytic phages, and stability of lytic phages at different buffer, temperature and pH were analyzed by standard deviation using Microsoft Excel.

## Results and Discussion

In the present study, out of seven sampling areas, only one area DWA3 showed the presence of *Salmonella* lytic phages (Table 1). *Salmonella* spp. were found in wastewater in several countries such as Mediterranian (Baudart *et al.,* 2000), Abidjan (Julien *et al.,* 2014), Nigeria (Dickson *et al.,* 2016), France, Spain, Finland, Morocco, Mexico and USA (Boulani *et al.,* 2017). The presence of four lytic phages that were capable of infecting *Salmonella enterica* was reported in approximately 66.67% of

**Table 1:** Sources of phage

| Sources of phage (area) | Amount of sample (x 100 ml) | Phage isolation results (host *Salmonella* spp.) | | |
|---|---|---|---|---|
| | | *Salmonella* S1 | *Salmonella* S2 | *Salmonella* S3 |
| DWA 1 | 3 | - | - | - |
| DWA 2 | 2 | - | - | - |
| DWA 3 | 2 | + (F-SB1) | + (F-SB2) | + (F-SB3) |
| DWA 4 | 3 | - | - | - |
| DWA 5 | 2 | - | - | - |
| DWA 6 | 2 | - | - | - |
| DWA 7 | 2 | - | - | - |

DWA: Domestic waste water area; (-) phages absent and (+) = phages present

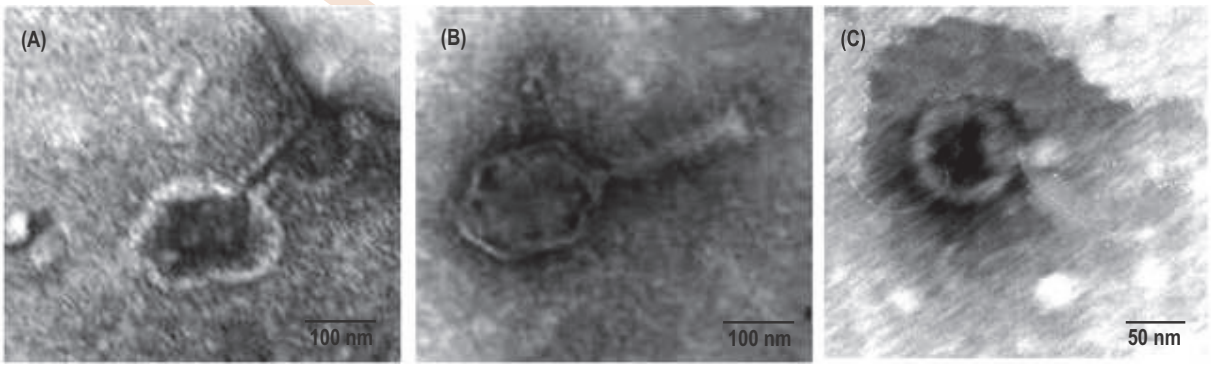


**Fig. 1 :** Morphology of phages: F-SB1 (A); F-SB2 (B) and F-SB3 (C)

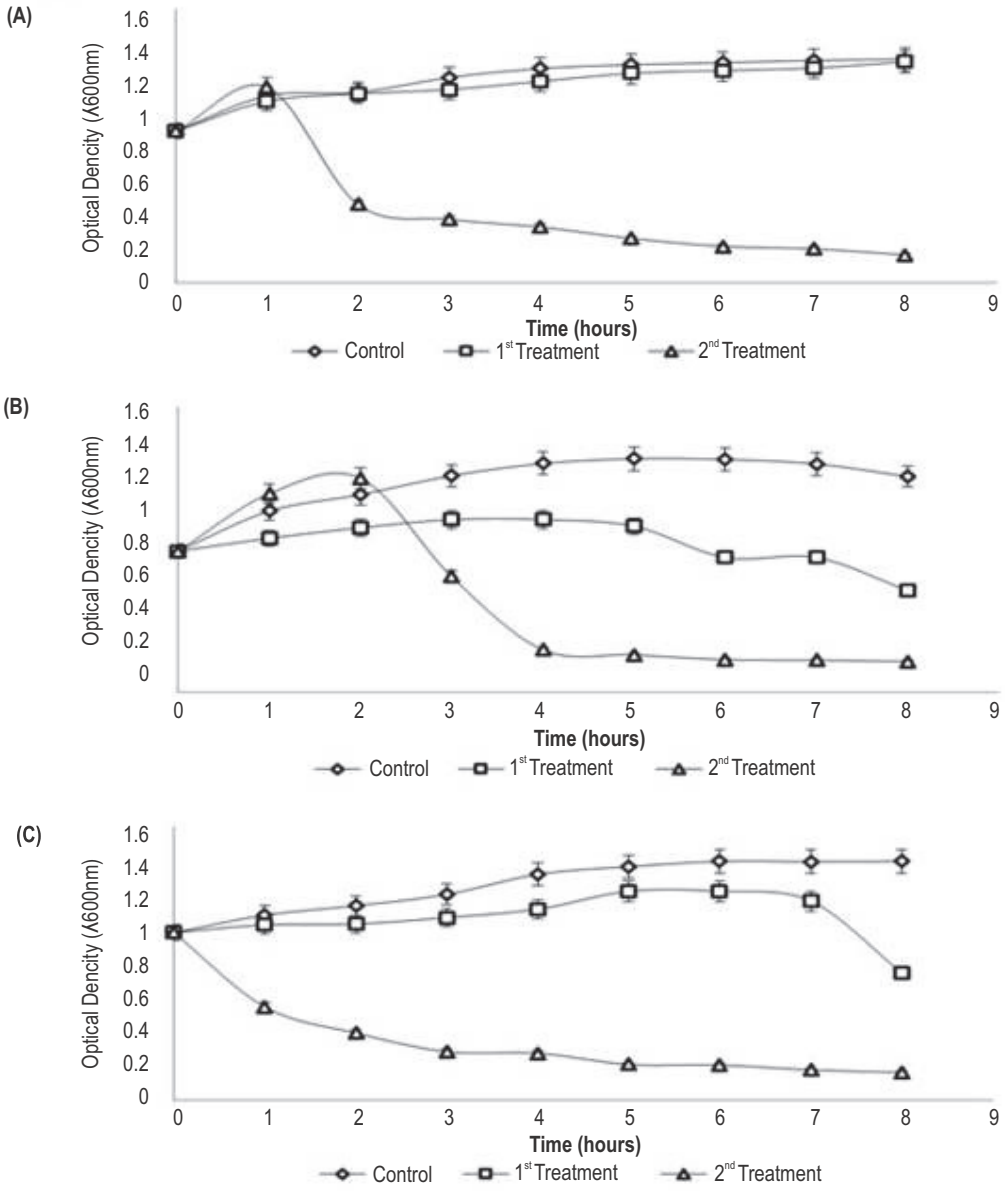


**Fig. 2 :** Effectiveness of lytic phages F-SB1, F-SB2, and F-SB3 to lyse *Salmonella* S1 (A), S2 (B), and S3 (C) cells incubated at 37 °C. Control: *Salmonella* spp. without phages, 1st treatment: *Salmonella* spp. + phages ($10^4$ pfu $ml^{-1}$), 2nd treatment: *Salmonella* spp. + phages ($3 \times 10^4$ pfu $ml^{-1}$).

wastewater from poultry processing industry in Pichincha-Ecuador (Quiroz *et al.,* 2016). Phages are abundant in aquatic environment and play an important role in controlling their host population (Doss *et al.,* 2017). Phages are only able to infect and reproduce in a suitable host, but they are not capable of replicating without a host cell. In Indonesia, it has been discovered that lytic phages can lyse pathogenic bacteria, *i.e., Bacillus pumilus* (Kusmiatun *et al.,* 2015), EPEC contaminated food and water (Arivo *et al.,* 2016) and *Staphylococcus aureus* (Nugroho *et al.,* 2016).

The presence of three lytic phages (F-SB1, F-SB2 and FSB3) in the samples were indicated by a clear zone or plaque formed in agar plate. The plaque formed from each *Salmonella* spp. isolate had different concentration and diameters. The concentration of phage F-SB1, F-SB2, and F-SB3 were 33600 pfu

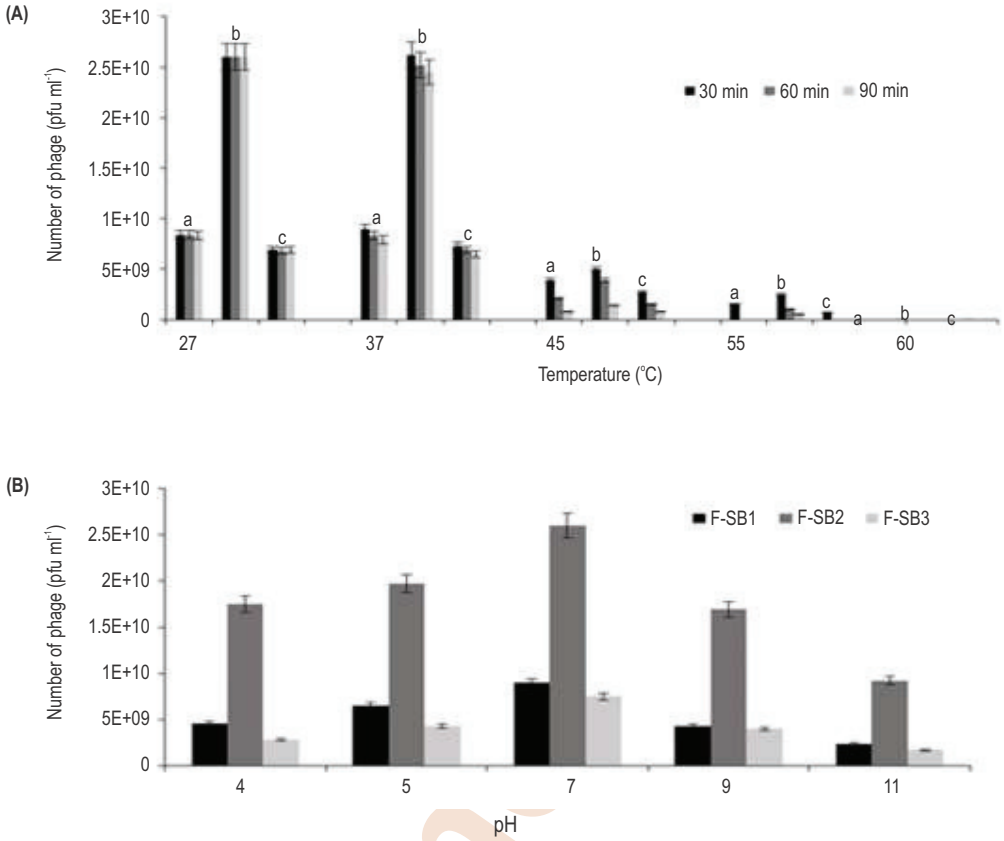


**Fig. 3 :** Stability of lytic phages. (A) at different temperatures (27 °C, 37 °C, 45 °C, 55 °C and 60 °C for 30 min, 60 min and 90 min. F-SB1 (a), F-SB2 (b) and F-SB3 (c); (B) at different pH for 30 min. All data are means of three replicates ± SD.

$ml^{-1}$, 11440 pfu $ml^{-1}$ and 10720 pfu $ml^{-1}$ respectively. Transmission electron microscope revealed the nano-sized isolated lytic phages which had several morphological types of head and tail (Fig. 1). Based on the morphological characteristics of the lytic phages (Fig. 1), phage F-SB1 with a long tail can be classified into family Siphoviridae, while phage F-SB2 with a tail surrounded by a sheath can be classified into Myoviridae (Ackermann, 2009). Phage F-SB3 can be classified into family Podoviridae and order Caudovirales based on the tail morphology, which was shorter compared to the other two phages (Fokine and Rossmann, 2014; Adriaenssens and Brister, 2017). The results of this research is in contrast with the results of Atterbury *et al.* (2007), who reported that *Salmonella* phages isolated from wastewater belonged to family Myoviridae and Siphoviridae.

After testing the effectiveness of phage infection, it was found that the three types of lytic phage were able to decrease the population of *Salmonella* spp. At $10^4$ pfu $ml^{-1}$, all the three phages were able to reduce the host population compared to the control. The time required to decrease the host population is different for each phage. By increasing the phage concentration ($3x10^4$ pfu $ml^{-1}$) in the second treatment, the decrease in host population was faster than the first treatment (Fig. 2). Interestingly, based on the results of this research, the phages may be more potential in decreasing the population of *Salmonella* spp. than the phages used in the study of Hungaro *et al.* (2013), where $10^8$-$10^9$ pfu $ml^{-1}$ concentration was used. It has been reported that phages can be used efficiently in modern biotechnology as an alternative to lyse many antibiotic resistant bacterial pathogens (Haq *et al.,* 2012; Wittebole *et al.,* 2013; Elbreki *et al.,* 2014; Quiroz *et al.,* 2016). In addition, previous researchers have reported that lytic phages can be employed as an alternative of bio-control agents to reduce contamination by *S. enterica* or *S. enteritidis* (Bao *et al.,* 2011; Thung *et al.,* 2016), EPEC $K1^{-1}$ (Arivo *et al.,* 2016), *B. pumilus* (Kusmiatun *et al.,* 2015) and foodborne pathogenic bacteria (Thung *et al.,* 2017).

In terms of effectiveness, different morphological characteristics of lytic phages indicate that they were able to infect the bacteria with different virulence numbers as shown in

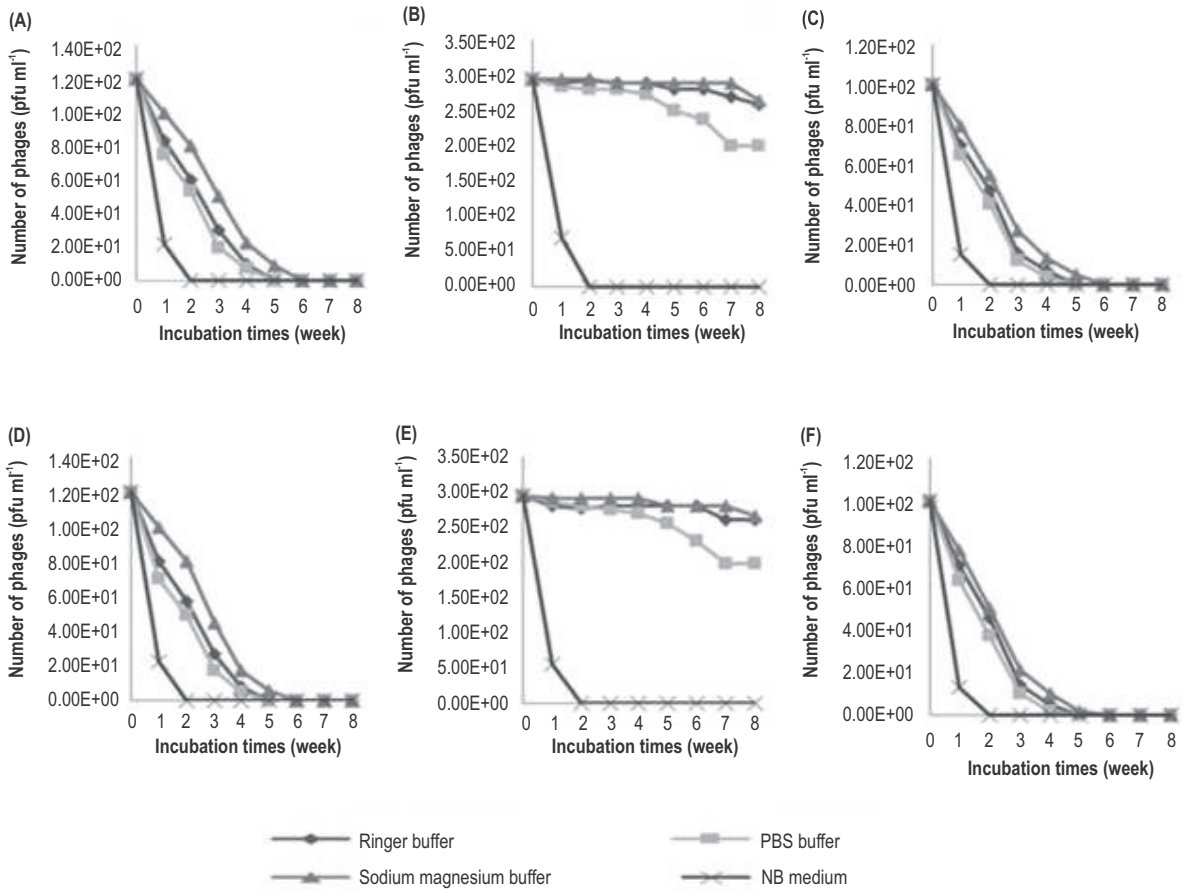


**Fig. 4:** Stability of lytic phages at different buffers at room temperature 27 °C; (A) F-SB1; (B) F-SB2; (C) F-SB3; at lower temperature 4 °C and (D) F-SB1; (E) F-SB2 and (F) F-SB3.

Atterbury's study. It was found that different morphological characteristics cause different virulence numbers in infecting *Salmonella* spp. (Atterbury *et al.*, 2007). The difference of morphological characteristics of the phages in this study may cause difference in their ability to penetrate host cells which would result in different effectiveness. This might be one of the reasons why each phage needs different time in decreasing the host population. It is known that lytic phages have different molecular protein weights of 16-42 kDa, 16-120 kDa and 12-57 kDa, respectively. (data not shown). Phage F-SB2 had the highest protein concentration (305 ug $ml^{-1}$), while phage F-SB1 had smaller protein concentration (268 ug $ml^{-1}$) than phage F-SB2. Phage F-SB3 had the lowest protein concentration (230 ug $ml^{-1}$) among all phages.

In terms of stability of lytic phages, temperature plays an important role in the stability of phage protein. Some proteins can be denatured at high temperatures, even at temperatures slightly above the optimum temperature (Siang *et al.*, 2004). The isolated lytic phages (F-SB1, F-SB2, and F-SB3) were stable at 27°C to 37°C after 30 min incubation, but they became unstable when the temperature is increased to 45°C, 55°C and 60°C (Fig. 3a). In addition, all phages, in this research, were stable at acidic pH (4-5) to neutral (6-7) pH, and pH 7 was considered as optimum (Fig. 3b).

The other physical and chemical factors that determine the stability of phage were pH and ion. Based on buffer assay, phage F-SB2 showed the best stability in sodium magnesium buffer at 27°C or 4°C, while two other phages F-SB1 and F-SB3 showed the best stability in sodium magnesium buffer at 27°C (Fig. 4). The difference in phage stability is influenced by the chemical composition contained in the buffer. Therefore, the damage on structural elements such as head, tail and DNA structure changes may inactivate phage due to inappropriate (Jończyk *et al.,* 2011).

All phages found in this research have different morphological characteristics and virulence numbers to decrease

the *Salmonella* population. They were almost identical in biological characteristics and stable at 27°C-37°C temperature, pH 4-7 and sodium magnesium buffer. Therefore, this study gives information in phage application as a biocontrol agents.

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## References

Ackermann, H.W.: Phage Classification and Characterization. In: (Eds.: M. R. Clokie and A. M. Kropinski), Bacteriophages Methods and Protocols. Vol. 501, p. 127. Humana Press, New York (2009).

Adriaenssens, E.M. and J.R. Brister: How to name and classify your phage: An informal guide. *Viruses,* **9**, 1–9 (2017).

Ao, T.T., N.A. Feasey, M.A. Gordon, K.H. Keddy, F.J. Angulo and J.A. Crump: Global burden of invasive nontyphoidal *Salmonella* disease, 20101. *Emerg. Infect. Dis.,* **21**, 941–949 (2015).

Arivo, D., I. Rusmana and S. Budiarti: Isolation and characterization of EPEC phage from domestic waste in Indonesia. *Malay. J. Microbiol.,* **12**, 140–146 (2016).

Atterbury, R.J., M.A.P. Van Bergen, F. Ortiz, M.A. Lovell, J.A. Harris, A. De Boer and P.A. Barrow: Bacteriophage therapy to reduce *Salmonella* colonization of broiler chickens. *Appl. Environ. Microbiol.,* **73**, 4543–4549 (2007).

Bao, H., H. Zhang and R. Wang: Isolation and characterization of bacteriophages of *Salmonella enterica* serovar Pullorum. *Poult. Sci.,* **90**, 2370–2377 (2011).

Baudart, J., K. Lemarchand, A. Brisabois and P. Lebaron: Diversity of *Salmonella* strains isolated from the aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. *Appl. Environ. Microbiol. Environ. Microbiol.,* **66**, 1544–1552 (2000).

Bertelloni, F., M. Chemaly, D. Cerri, F. Le Gall and V.V. Ebani: *Salmonella* infection in healthy pet reptiles: Bacteriological isolation and study of some pathogenic characters. *Acta Microbiol. Imm. H.,* **63**, 203-216 (2016).

Boulani, A.R.El, Mimouni, E. Boulani and R. Mimouni: *Salmonella* in wastewater: Identification, antibiotic resistance and the impact on the marine environment. In: Current topics in *Salmonella* and salmonellosis. INTECH, World's largest Science, Technology & Medicine Open Access Book Publisher, pp.137-148 (2017).

Budiarti, S. Y. Hirai, J. Minami, S. Katayama, T. Shimizu and A. Okabe: Adherence to HEp-2 cells and replication in macrophages of *Salmonella derby* of human origin. *Microbiol. Immunol.,* **35**, 111-123 (1991).

Dickson, D.I., I.A. Chibuogwu and E.I. Maureen: Serotyping and molecular typing of *Salmonella* species isolated from wastewater in Nsukka , Nigeria. *Afr. J. Microbiol. Res.,* **10**, 883–889 (2016).

Dinkar, N., A.K. Sharma, A.S. Ninawe and Y. Prasad. Molecular characterization of lytic phages specific to antibiotic resistant isolates of *Pseudomonas fluorescens* infecting *Labeo rohita* and *Clarias batrachus* of Sub Himalayan region. *J. Environ. Biol.,* **38**, 353-360 (2017).

Doss, J., K. Culbertson, D. Hahn, J. Camacho and N. Barekzi: A review of phage therapy against bacterial pathogens of aquatic and terrestrial organisms. *Viruses,* **9**, 1–10 (2017).

Ejo, M., L. Garedew, Z. Alebachew and W. Worku: Prevalence and antimicrobial resistance of *Salmonella* isolated from animal-origin food items in Gondar, Ethiopia. *BioMed. Res. Int.,* 2016, 1–8 (2016).

Elbreki, M., R.P. Ross, C. Hill, J. O'Mahony, O. McAuliffe, A. Coffey and A. Coffey: Bacteriophages and their derivatives as biotherapeutic agents in disease prevention and treatment. *J. Virus.,* 2014, 1–20 (2014).

Eng, S.K., P. Pusparajah, N.S. Ab Mutalib, H.L. Ser, K.G. Chan and L.J. Lee: *Salmonella*: A review on pathogenesis, epidemiology and antibiotic resistance. *Front. Life. Sci.,* **8**, 284-293 (2015).

Fokine, A. and M.G. Rossmann: Molecular architecture of tailed double-stranded DNA phages. *Bacteriophage,* **4**, e28281 (2014).

Guenther, S., D. Huwyler, S. Richard and M.J. Loessner: Virulent bacteriophage for efficient biocontrol of *Listeria monocytogenes* in ready-to-eat foods. *Appl. Environ. Microbiol.,* **75**, 93–100 (2009).

Haq, I.U., W.N. Chaudhry, M.N. Akhtar, S. Andleeb and I. Qadri: Bacteriophages and their implications on future biotechnology: A review. *Virol. J.,* **9**, 9 (2012).

Hoelzer, K., A.I.M. Switt and M. Wiedmann: Animal contact as a source of human non-typhoidal salmonellosis. *Vet. Res.,* **42**, 34 (2011).

Hungaro, H.M., R.C.S. Mendonça, D.M. Gouvêa, M.C.D. Vanetti and C.L. Pinto de O. Use of bacteriophages to reduce *Salmonella* in chicken skin in comparison with chemical agents. *Food Res. Int.,* **52**, 75–81 (2013).

Jończyk, E., M. Kłak M, R. Międzybrodzki and A. Górski: The influence of external factors on bacteriophages. *J. Folia. Microbiol.,* **56**, 191–200 (2011).

Julien, C.K., G. Alahou, A. Gabazé, K. Kouamé, K.K. Stephane, Y.O. Bernard and D. Mireille: The antibiotic resisting profile of *Salmonella* spp. isolated from the sewage of the campus of the University of Cocody, Abidjan, Côte d' Ivoire. *Int. J. Trop. Dis. Health,* **4**, 608–620 (2014).

Kuhn, K.G., G. Falkenhorst, T.H. Ceper, T. Dalby, S. Etheberg, K. Molbak and K.A. Krogfelt: Detecting non-typhoid *Salmonella* in humans by ELISAs: a literature review. *J. Med. Microbiol.,* **61**, 1-7 (2012).

Kurek A.J. T. Gasior, B. N. Falenczyk, S. Bloch, A. Dydeca, G. Topka, A. Necel, M. Jakubowska-Deredas, M. Narajczyk, M. Richert, A. Mieszkowska, B. Wrobel, G. Wegrzyn and A. Wegrzyn: Biodiversity of bacteriophages: morphological and biological properties of a large group of phages isolated from urban sewage. *Sci. Reports.,* **6**, 34338 (2016).

Kusmiatun, A., I. Rusmana and S. Budiarti: Characterization of bacteriophage specific to *Bacillus pumilus* from Ciapus River in Bogor, West Java, Indonesia. *HAYATI J. Biosci.,* **22**, 27–33 (2015).

Kusumaningrum, H. D., Suliantari and R. Dewanti-Hariyadi: Multidrug resistance among different serotypes of *Salmonella isolates* from fresh products in indonesia. *Int. Food Res. J.,* **19**, 57–63 (2012).

Laemmli, U.K: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* **227**, 680–685 (1970).

Majowicz, S.E., J. Musto, E. Scallan, F.J. Angulo, M. Kirk, S.J. O'Brien and R.M. Hoekstra: The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin. Infect. Dis.,* **50**, 882–889 (2010).

Matias, C.A.R., I.A. Pereira, M. Araújo, dos S. de, A.F.M. Santos, R.P. Lopes, S. Christakis and S. Siciliano: Characteristics of *Salmonella* spp. isolated from wild birds confiscated in illegal trade markets, Rio de Janeiro, Brazil. *BioMed. Res. Int.,* 2016, 1–7 (2015).

Miyakawa, S., K. Takahashi, M. Hattori, K. Itoh, T. Kurazono and F. Amano: Outbreak of *Salmonella* oranienburg infection in Japan. *J. Environ. Biol.,* **27**, 157-158 (2006).

Nugroho, P.D., S. Budiarti and I. Rusmana: Characterization of lytic phage *Staphylococcus aureus* from dairy farm cows in Indonesia. *Int. J. PharmTech. Res.,* **9**, 10–18 (2016).

Prasad, Y., Arpana, D. Kumar and A.K. Sharma: Lytic bacteriophages

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specific to *Flavobacterium columnare* rescue catfish, Clarias batrachus (Linn.) from columnaris disease. *J. Environ. Biol.,* **32**, 161-168 (2011).

Quiroz, E., J. Recalde, M.T. Arias, R. Seqqat, C. Vinueza and L. Ayala: Isolation of lytic bacteriophages for nanobiocontrol of pathogenic and antibiotic resistant *Salmonella* present in poultry in Ecuador. *Biol. Med.,* **8**, 1–6 (2016).

Sartika, D., S. Budiarti and M. Sudarwanto. Phage FR38 treatment on sprague dawley rat inferred from blood parameters and organ systems. *HAYATI J. Biosci.,* **19**, 131–136 (2012).

Siang, T.W., P. Ramanujam and S. Nathan: Effect pH and temperature on the binding of a fusion phage to newcastle disease virus. *J. Biochem.,* **9**, 35-37 (2004).

Thung, Tze Young, JMKJ Premarathne, W.S. Chang, Y.Y. Loo, Y.Z. Chin, C.H. Kuan, C.W. Tan, D.F. Basri, C.W.J.W.M. Radzi and S. Radu: Use of a lytic bacteriophage to control *Salmonella enteritidis* in retail food. *Food Sci. Technol.,* **4**, 1–4 (2016).

Thung, T.Y., B.M.F. Siti Norshafawatie, J.M.K.J.K. Premarathne, W.S. Chang, Y.Y. Loo, C.H. Kuan and R. Son: Isolation of food-borne pathogen bacteriophages from retail food and environmental sewage. *Int. Food Res. J.,* **24**, 450–454 (2017).

Tjanadi, P., M. Lesmana, D. Subekti, N. Machpud, S. Komalarini, W. Santoso and B. A. Oyofo: Antimicrobial resistance of bacterial pathogens associated with diarrheal patients in Indonesia. *Am. J. Trop. Med. Hyg.,* **68**, 666–670 (2003).

Toroglu, S., E. Toroglu, S. Dincer, C. Kara and M. Kertmen: Resistances of antibiotics and heavy metals in *Enterobacteriaceae* spp. isolated from gills and intestines of *Achanthobrama marmid* (Heckel, 1843) from Sir Dam lake Turkey. *J. Environ. Biol.,* **30**, 23-31 (2009).

Turki, Y., H. Ouzari, I. Mehri, Ammar, A. Ben and A. Hassen: Evaluation of a cocktail of three bacteriophages for the biocontrol of *Salmonella* of wastewater. *Food Res. Int.,* **45**, 1099–1105 (2012).

Wittebole, X., S. De Roock and S.M. Opal: A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. *Virulence,* **5**, 226–235 (2013).

Zuber, S., C. Boissin-Delaporte, L. Michot, C. Iversen, B. Diep, H. Brüssow and P. Breeuwer: Decreasing *Enterobacter sakazakii* (*Cronobacter* spp.) food contamination level with bacteriophages: Prospects and problems. *Microbial. Biotechnol.,* **1**, 532–543 (2008).

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