JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

PARTIAL CHARACTERIZATION OF BACTERIOPHAGES INFECTING *SALMONELLA* SP. CAUSE OF FOODBORNE DISEASE

*Erlia Narulita[*1,2,4], Fiqih Ramadhan[3], Riska Ayu Febrianti[2,4], Ria Yulian[5], Afifatur Rofiqoh[2], Zidna Amalia Firdausy[2], Mochammad Iqbal[1]*

Address(es):
[1] Department of Biology Education, University of Jember. Jl. Kalimantan No. 37, Tegalboto, Jember 68121, East Java, Indonesia.
[2] Laboratory of Molecular Medicine, Center for Development of Advanced Sciences and Technology, University of Jember. Jl. Kalimantan No. 37, Tegalboto, Jember 68121, East Java, Indonesia.
[3] Division of Life Science, Graduate School of Life Science, Hokkaido University, Sapporo 060-0810, Hokkaido, Japan.
[4] Department of Biotechnology, Postgraduate Program, University of Jember. Jl. Kalimantan No. 37, Tegalboto, Jember 68121, East Java, Indonesia.
[5] Department of Agricultural Biological Chemistry, College of Agriculture and Life Sciences, Chonnam National University, Gwangju 500-757, South Korea.

*Corresponding author: erlia.fkip@unej.ac.id

https://doi.org/10.55251/jmbfs.10240

ARTICLE INFO

Received 3. 6. 2024
Revised 12. 2. 2025
Accepted 14. 2. 2025
Published 1. 4. 2025

Regular article

OPEN ACCESS

ABSTRACT

Salmonella sp. resistance against various antibiotics is one of the current health problems. An alternative to dealing with *Salmonella* sp. is using a bacteriophage-based biocontrol. In this study, the isolation of bacteriophages originated from Kencong and Puger areas in Jember Regency East Java, which was prone to food poisoning cases. Eleven bacteriophages were isolated from washing-water samples (φSC1, φSC2, φSC3, φSC4, φSC5), food samples (φSM1, φSM2, φSM3), and fish-waste samples (φSUT, φSP1, φSP2). The result showed that φSC3, φSC4, φSC5, and φSM1 have broad infection capabilities to the genera of *Salmonella, Escherichia*, and *Staphylococcus*. All isolates were classified as bacteriophages with DNA nucleic acids. The TEM morphological observations indicated that φSC4 isolates belong to the Podoviridae while φSP1 isolates belong to the Myoviridae. These results suggest that the phages could potentially be used for biocontrol purposes. However, further characterization, viability measurement and activity are needed before their use in food applications against foodborne pathogens.

Keywords: Bacteriophage, foodborne disease, *Salmonella* sp.

INTRODUCTION

Foodborne disease is mostly caused by contamination during the process of food preparation or serving. Contamination occurs through water, dust, air, soil, or food processing equipment (**Ameme *et al.*, 2016**). Nowadays, foodborne diseases become one of the global health problems (**Bhardwaj *et al.*, 2015**). In Kencong Area, a district of Jember Regency East Java Indonesia, in 2016, there were 225 positive cases of food poisoning. Even until March 2017, there were still positive cases in the area. In general, two out of three cases of food poisoning are caused by bacteria (**Yang *et al.*, 2013**). The causative agents of foodborne diseases can generally be classified into pathogenic bacteria (*Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Cronobacter sakazakii, Esherichia coli, Listeria monocytogenes, Salmonella* spp., *Shigella* spp., *Staphylococccus aureus*, and *Vibrio siniapp*, viruses (Hepatitis A and Noroviruses) and parasites (*Cyclospora cayetanensis, Toxoplasma gondii*, and *Trichinella spiralis)* (**Bintsis, 2017**). Based on **Nadi *et al.* (2020)** Salmonella are the most common species found in foodborne diseases and their presence does not depend on the location, season, and the level of primary health of a country.

The Salmonella-related disease is gaining more attention due to increasing incidents in Indonesia. Typhoid fever occurrence, for example, it reaches 148.7 per 100,000 people/year (**Ochiai *et al.*, 2008**). The emergence of multidrug resistance of Salmonella to almost all antibiotics causes a health crisis. Not only increasing medical costs but also increase the percentage of fatality (**Faruk *et al.*, 2014**). Salmonella sp. causing 3 million people died of typhoid fever with the Asian continent occupying the highest percentage of deaths by 80% (**Doffkay *et al.*, 2015; Acosta *et al.*, 2017**). Endemic countries and at high risk for this disease are Pakistan, India, China, Bangladesh, Indonesia, Malaysia, Vietnam, and Tajikistan (**Ochiai *et al.*, 2008; Iqbal *et al.*, 2020**).

Therapies and treatments that are often used to treat *Salmonella* infections are using antibiotics. Antibiotics can solve these problems, but will create a new problem called bacterial antibiotic resistance. According to **Herrera-Sánchez *et al.* (2021)** some strains of *Salmonella* are resistant to fluoroquinolones due to the presence of a resistant gene called qnrB in their plasmid. Research Wain *et al* (2021) describes the resistance of Salmonella to ceftriaxone. Based on **Patra *et al.* (2021)** *Salmonella enterica Typhimurium* in South Asian countries (including Malaysia, Thailand, Vietnam, Indonesia, Cambodia, Singapore, and the Philippines) is resistant to three or more groups of antibiotics (multidrug resistance). Therefore, the use of antibiotics is not effective to overcome this problem.

Another treatment for bacterial infections is the use of bacteriophages. Bacteriophages are viruses that specifically infect certain species or even specific strains of bacteria (**Kakasis & Panitsa, 2019**). The results of the study of bacteriophage treatment on *Salmonella* by **Lamy-Besnier *et al.* (2021),** using mice, proved safe and efficient. They used two types of bacteriophages to infect *Salmonella typhimurium*. Bacteriophages delay and reduce *Salmonella thyphimurium* infection in mice. Host specificity directs the bacteriophage to minimize the impact on the normal flora in the human body.

This character attributes to the advantages of using bacteriophages as biocontrol. It does not harm non targeted cells, has a low potential for resistance and high stability. Bacteriophages possess a high ability to mutate and replicate and able to adjust to the number of their specific target bacteria (**Putra and Karuniawati 2012; Haq *et al.*, 2012; Taj *et al.*, 2014; Susianto *et al.*, 2014**). Hence it can be used to treat chronic bacterial infections. Another use of bacteriophages is as an anti-biofilm agent (**Abedon, 2005**), disinfectant for the medical environment a detector kit for growth and development of pathogenic bacteria, as well as antibacterial therapy for pathogens (**Brussow, 2005; Skurnik *et al.*, 2006; Mangieri *et al.*, 2020).**

Bacteriophage therapy has become a center of attraction for biomedical scientists to treat foodborne diseases caused by pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa*, and *Salmonella* sp. (**Singla *et al.*, 2016; Bai *et al.*, 2016; Narulita *et al.*, 2018). Many studies of bacteriophage as biocontrol of *Salmonella* sp. have been carried out (Sillankorva *et al.*, 2012; Bao *et al.*, 2015; Bardina *et al.*, 2016; Malik *et al.*, 2017).** However, the study of bacteriophages as a biocontrol of *Salmonella* sp. is still rarely done in Indonesia.

Phage therapy has been proven effective in controlling the pathogens that cause food poisoning, both pre-harvest and post-harvest. The provision of phage in the pre-harvest and post-harvest can control bacterial contamination such as *E. coli*

O157: H7, *Salmonella*, and *Campylobacter* in food products. However, bacteriophages cannot be used directly as biocontrols because the characterization and interaction between bacteriophages and host bacteria that are not yet clear will increase host virulence (**Tarabees *et al*., 2016**). Thus, molecular characterization is needed to determine the diversity of bacteriophage *Salmonella* sp. and to analyze interactions between phage and host bacteria. The objectives of this paper were to virulence ability of bacteriophages infecting *Salmonella* spp. by host range analysis and partial characterization of bacteriophage such as the morphological structure of bacteriophages, nucleic acid content, and protein profiles.

MATERIAL AND METHODS

Isolation and characterization of Bacterial Host Strains

Salmonella sp. isolated from two food samples of street vendors in Kencong and one sample from Puger fish auction market, Jember. The samples obtained were enriched using Luria Bertani Broth and incubated for 24 hours. Enriched samples were diluted, and serial dilutions of 10-3 were planted on Salmonella Shigella Agar (SSA). A single colony was purified using the quadrant streak plate method (**Yamada *et al*., 2007**).

Isolation and Purification of Bacteriophages

Same sampel using for isolation *Salmonella* sp. also used for isolation bacteriophages. The sampels were cultured on Luria Bertani (LB) Broth, which had been inoculated with *Salmonella* sp. for 24 hours. The incubation results are centrifuged at 12,000 rpm for 10 minutes. The supernatant is filtered using a 0,22 µm membrane filter. The filtration results were then conducted for a spot test on *Salmonella* sp., which will serve as the primary host. Bacteria that showed positive results on the spot test then were tested plaque. The plaque that arises is taken and cultured on LB Broth. Centrifugation was carried out at 12,000 rpm for 10 minutes. The supernatant obtained was filtered using a membrane filter. The filter results are pure bacteriophage isolates that are ready to use. Back culture was performed using the spot test method, which was then shaken with the SM Buffer to extend the life of the bacteriophage sample. Bacteriophage isolates in BC Buffer are used as work isolates (**Ackermann, 2009**).

Host Range Analysis of Bacteriophages

Host range analysis was performed using the spot test method from **Narulita *et al* (2018)** with slight modification of titer bacteriophage droplets on top agar using 108 PFU/mL. The bacteria used in this test can be seen in Table 1. The bacteria to be used were cultured on Luria Bertani Broth then incubated at 37ºC for 5 hours (initial exponential phase). The incubated bacterial culture was included in Top Agar (0.8% agar) as much as 300 µL and poured in Luria Agar. The double-layer medium that has been made is then allowed to stand for about 30 minutes, and then it is filtered with several bacteriophages tested to determine the host range. Each bacteriophage was deposited as much as 5 µL.

Preparation of bacteriophages for transmission electron microscopy

The highest titer of bacteriophages in the liquid medium obtained from the primary host of each bacteriophage, was then purified. The concentrated phage was dripped on a copper grid coated with formvar-carbon and allowed to absorb for 2 minutes. Staining was done using 1% Na-phosphotungstate and observed under an JEM-1400 JEOL/EO transmission electron microscope **(Narulita *et al*. 2016**).

Table 1 Bacteria used in this study

No	Bacteria Name / Isolate Code of Isolate	Origin
1.	*Salmonella* spp./ KP1	Food, Food, Kencong
2.	*Salmonella* sp. / KP2	Yulian *et al*. (2020)
3.	*Salmonella* spp./ KJ12	Food, Kencong
4.	*Salmonella* spp./ P21A	Fish, Puger
5.	*Salmonella* spp ./ P21B	Fish, Puger
6.	*Salmonella* sp./ P21D	Yulian *et al*. (2020)
7.	*Salmonella. typhimurium* ATCC 14068	Lab. Molecular Medicine, CDAST
8.	*Salmonella typhi*	Lab. Microbiology, Department of Biology
9.	*Escherichia coli*	Lab. Microbiology, Department of Biology
10.	*Staphylococcus aereus*	Lab. Microbiology, Department of Biology

Bacteriophages Genomic Analysis

The standard technique for DNA isolation is that bacteriophage particles added with buffer lysis and Proteinase-K then added 1: 1 (v/v) PCI (25: 24: 1). The top layer was transferred into a new tube and added 3M Sodium acetate (10% × sample volume) and added 2.5× volume of absolute EtOH The mixture was incubated at -20°C overnight. The pellet was centrifuged at a speed of 15,000 rpm, 4ºC for 15 minutes. The pellets obtained were added with 500 µl 70% ethanol and centrifuged for 5 minutes, the fan then was dried. In the final stage, TE buffer was added pH 8. Before being visualized using horizontal electrophoresis of 3 µl, each genome that had been isolated was treated by adding DNAse and RNAse enzymes to find out the type of nucleic acids (**Sambrook and Russell, 2001**).

Structural Protein Profiles of bacteriophage

Analysis structural prorein profile determine using SDS-PAGE method was performed by Narulita *et al* (2018). A sample of 50 µL purified phage particles (5×1010 PFU/mL) was dissolved in 50 µL loading buffer (50 µL Mercaptoethanol, 950 µl sample buffer (2×) for SDS-PAGE). After heating at 95°C for 5 min, the samples were subjected to electrophoresis in 12% SDS-PAGE gel along with protein markers with Trisglycine as running buffer. After electrophoresis, proteins were visualized by staining with Coomassie Brilliant Blue.

RESULTS AND DISCUSSION

Isolation and purification bacteriophage

Table 2 showed morphological characteristics of each bacteriophage isolate. The data also showed primary host bacteria each bacteriophage and sample were origin. There were eleven bacteriophages obtained, five from washing water samples (φSC1, φSC2, φSC3, φSC4, φSC5), three from food samples (φSM1, φSM2, φSM3), one isolate from shrimp (φSUT) and 2 isolated from fish (φSP1 and φSP2). On average, each isolate has a size of more than 1 mm.

Table 2 Morphological Characteristics of Bacteriophages Infecting *Salmonella* sp.

No	Bacteriophage	Primary Host	Origin	Diameter Plaque (mm)	Characteristic of Plaque
1.	φSC1	KP1	Food, Kencong	± 2:01 - 3:05	Clear with no halo
2.	φSC2	KP1	Food, Kencong	± 2:23 - 3:10	Clear with no halo
3.	φSC3	KP2	Food, Kencong	± 2:14 - 3:18	Clear with no halo
4.	φSC4	KJ12	Food, Kencong	± 2.02 - 2.10	Clear with no halo
5.	φSC5	KJ1.2	Food, Kencong	± 2.12 - 3.12	Clear with no halo
6.	φSM1	KP1	Food, Kencong	± 2.04 - 3.08	Clear with no halo
7.	φSM2	KP2	Food, Kencong	± 2.07 - 3.80	Clear with no halo
8.	φSM3	KJ1.2	Food, Kencong	± 2.45 - 3.87	Clear with no halo
9.	φSUT	P21A	Fish, Puger	± 1.00 - 2.00	Halo
10.	φSP1	P21D	Fish, Puger	± 1.00 - 2.00	Clear with no halo
11.	φSP2	P21D	Fish, Puger	± 1.00 - 2.00	Halo

C: bacteriophage isolated from washing water; M: bacteriophage isolated from food; UT: bacteriophages isolated from shrimp; PI: bacteriophages isolated from fish

Host Range Test of Bacteriophage

The results of the host range test showed that four isolates of bacteriophage φSC3, φSC4, φSC5, and φSM1 could infect all bacteria used. These results indicate that the four bacteriophages belong to broad host range phage (Table 3), while other bacteriophage isolates cannot infect certain bacteria.

Table 3 Host Range Test of Bacteriophages Against Multiple Bacterial

Phage	Host Primary	Infections Range									
		KP1	KP2	KJ12	P21A	P21B	P21D	*S. typhimurium* ATCC 14 068	*S. typhi*	*E. coli*	*S. aereus*
φSC1	KP1	++	+	+	+	+	+	+	+	+	-
φSC2	KP1	++	-	+	+	++	+	+	+	-	-
φSC3	KP2	+	++	+	+	+	+	+	+	+	+
φSC4	KJ1.2	+	+	++	+	+	+	+	+	+	+
φSC5	KJ1.2	+	+	++	+	+	+	+	+	+	+
φSM1	KP1	++	+	+	+	+	+	+	+	+	+
φSM2	KP2	-	++	+	++	+	+	+	-	-	+
φSM3	KJ1.2	-	+	++	-	+	+	+	+	+	+
φSUT	P21A	-	+	-	++	+	+	+	+	-	+
φSP1	P21D	-	-	+	+	+	++	+	+	-	-
φSP2	P21D	-	-	+	+	++	++	+	+	+	-

++ (clear plaque), + (turbid plaque), - (not infected)

TEM Analysis of Bacteriophage

The results of TEM φSC4 showed the characteristics belongs to Podoviridae and showed a head size of 74x72 nm (Figure 1a). φSP1 is a classified as Myoviridae. The capsid is icosahedral, 75x70 nm in size (Figure 1b-1). The tail is extensive, 185 × 8.75 nm (Figure 1b-2) and has fibers tail in the terminal tail (Figure 1b-3).

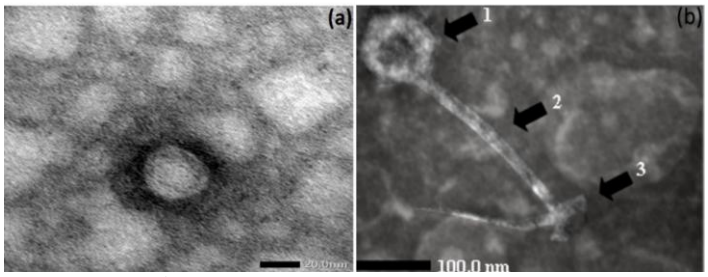

Figure 1 TEM of observation. (a) φSC4; (b) φSP1: capsid (black narrow 1), contractile (black narrow 2), fiber tail (black narrow 3). (a) scale bar 20 µm, (b) scale bare 100 µm.

Bacteriophage Genomic Analysis

Eight of eleven isolates bacteriophage succeed were genome isolated. In figure 2 showed all bacteriophage grouped as dsDNA. It can be seen from the results of genome treatment that DNAse successfully degraded the genome but not successful degraded by RNAse. The size genome from undigest with nuclease enzyme about > 1 kb.

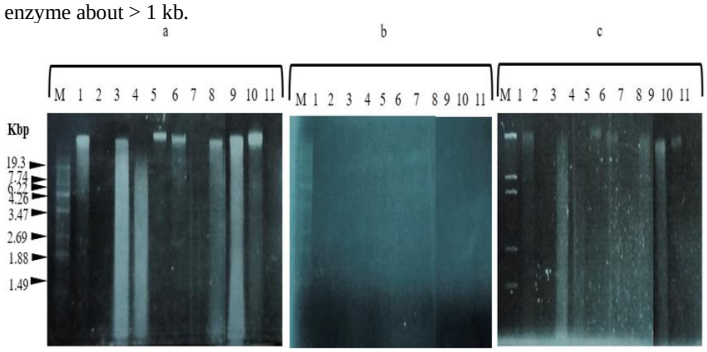

Figure 2 Visualization electrophoresis DNA genome of bacteriophage. (M) Marker λ/StyI, (1) φSC1, (2) φSC2, (3) φSC3, (4) φSC4, (5) φSC5, (6) φSM1, (7) φSM2, (8) φSM3, (9) φSUT, (10) φSP1, (11) φSP2. (a) genome bacteriophage undigest/without treatment with DNAse/RNAse, (b) genome bacteriophage treatment with DNAse, (c) genome bacteriophage treatment with RNAse.

Structural Protein Profiles

Three bacteriophage from Puger fish auction market succeed determination profiling structural protein. Bacteriophage φSUT has one major protein that is 44 kDa and has minor proteins of 170 kDa, 120 kDa, 64 kDa, 37 kDa. Bacteriophage φSP1 has one major protein measuring 34 kDa and minor proteins measuring 52 kDa and 47 kDa. Bacteriophage φSP2 has one major protein measuring 34 kDa and minor proteins measuring 57 kDa, 48 kDa, 42 kDa (Figure 3).

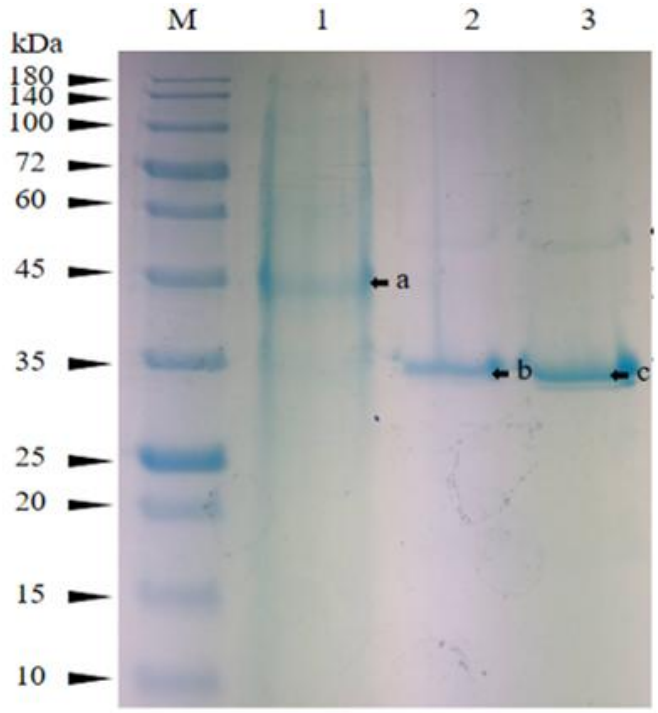

Figure 3 Visualization electrophoresis profiling protein bacteriophage by SDS-PAGE. (M) protein marker, (1) φSUT, (2) φSP1, (3) φSP2. black narrow (a) indication mayor protein of φSUT = 44 kDa, black narrow (b) indication mayor protein of φSP1 = 34 kDa, black narrow (c) indication mayor protein of φSP2 = 34 kDa.

DISCUSSION

Bacteriophages or phages are viruses that infect bacteria. Based on **Kwiatek *et al* (2020)**, the ability to infect and destroy bacterial cells makes bacteriophages a candidate for bacterial infection therapy. Bacteriophage therapy has many advantages and can overcome antibiotic problems, such as antibiotic resistance. Bacteriophages perform specific activity against target pathogens, without reducing or eliminating other physiological microflora. The immune system will also eliminate bacteriophages from the body after eradicating the target pathogen. Bacteriophages exist in every environment as long as a bacterial host is available and plays an important role in many biological processes **(Kakasis & Panitsa, 2019)**. In this study, the isolation of bacteriophages originated from Kencong and Puger areas in Jember Regency East Java, which was prone to food poisoning cases.

Plaque grouped into three sizes: small if the plaque is less than 2 mm, medium if the plaque is 2 mm, large if plaque-sized is more than 2 mm (**Ellis and Winters, 1969; Melo *et al*., 2020**). From this reference, isolate bacteriophages from Kencong grouped in medium until large plaque and isolate bacteriophages from Puger grouped in small plaque. The three bacteria isolated from Kencong and Puger can be the primary host of bacteriophages isolated from the same location. It is because the isolated phages have receptors that interact and infect these bacteria, which allows the formation of plaque. Receptors with different structures will not be possible for interaction, so there will be no plaque formed (**Haq *et al*., 2012**). There was no significant difference in bacteriophage type between samples from wastewater, food, shrimp, and fish. Based on the size of bacteriophages with an average size of 2 mm for each bacteriophage isolate, the Myoviridae type was found to be more dominant.

The plaque which appeared in the eighth isolates from Kencong belongs to the lytic bacteriophage (virulent), no bacteriophages classified as lysogenic (temperate). On the other hand, more diverse results showed in isolates from Puger. φSUT a very turbid plaque, φSP1 formed not turbid and not clear plaque, and φSP2 formed apparent plaque. Highly virulent or lytic strains that create plaques that look clear is apparent plaque, while strains that only kill a fraction of their hosts, or only reduce the rate of cell growth is turbid plaque (Ko¨hler et al., 2010). Based on their life strategy, phages are divided into moderate phages and virulent phages. Phages are undergoing horizontal gene transfer to propagate their virulence genes. Virulent phages will lyse their hosts and are an ideal weapon to destroy bacterial cells (**Kutter & Sulakvelidze, 2005**). According to the type of infection, bacteriophages are classified into two types: lytic and lysogenic. Lytic bacteriophages (also known as virulent bacteriophages) can cause death in host bacterial cells due to the process of lysis and release of new phages into the extracellular space. Lysogenic bacteriophages (also known as medium bacteriophages) will integrate genetic material into the host bacterial cell genome and will be replicated along with the host bacterial genome without a lysis mechanism (**Gordillo Altamirano & Barr, 2019**). Phage therapy is defined as the administration of virulent phages directly to patients with the aim of lysing pathogenic bacteria that cause clinically relevant infections (**Clokie *et al*., 2011**).

In contrast, lysogenic bacteriophages only integrate nucleic acids in host cells. So that bacterial host cells can move (**Samson *et al*., 2013).** Besides, turbid plaque also occurs due to the imperfect lysis process because of weak adsorption levels on the host cell. It produces immature bacteriophage particles in the lytic cycle (**Bondy-Denomy *et al*., 2016; Kawasaki *et al*., 2016; Weinbauer, 2004**). Other factors that affect the shape of the resulting plaque include a decrease in the lytic level of a bacteriophage in infecting host cells (lysis inhibitory phenomenon), differences in the ability of bacteriophages to produce specific enzymes to destroy bacterial envelope that result in plaque appearance (**Narulita *et al*. 2016**).

Generally, bacteriophages can only infect a narrow range of cells or the host specific (**Narulita *et al*. 2016; Grygorcewicz *et al*., 2015; Adam 1959**). It occurs due to a combination of several factors, including the incompatibility of bacteriophage binding proteins in bacterial receptors, biochemical interactions during the infection process, the presence of associated prophages (mainly in bacteriophages that absorb into the pili), and resistance mechanisms in bacteriophages (**Hyman and Abendon, 2010; Diaz-Munoz.and Koskella, 2014; Golkar *et al*., 2014; Leskinen *et al*., 2017**). The results of the host range test showed that the eight isolates had broad range capability, with four bacteriophage isolates (φSC3, φSC4, φSC5, and φSM1) infect all bacteria used in the host range test. The presence of broad range bacteriophages is because these bacteriophages have many protein binding receptors on their capsid, which provide the ability of at least two proteins to adsorb and recognize different structures in bacteria (**Santos *et al*., 2011**). In addition, the broad range capability of bacteriophages is also caused by the presence of lytic enzymes and endolysin enzymes (**Legotsky *et al*., 2014; Shende *et al*., 2017**).

Bacteriophage phenomenon *Salmonella* spp. which has a wide host was reported in several studies. Bacteriophage EHR1 can infect *S. aureus*, *Salmonella* spp., *P. aeruginosa*, *Klebsiella* spp., and *Proteus* spp. (**Jurczak-Kurek *et al*., 2016**); φS-394 is capable of infecting gram-negative bacteria Enterobacteriaceae (**Samson *et al*., 2013**); rV5-like phage has a range of infections covering many gram-negative Enterobacteriaceae (**Kropinski, 2009**); Felix-O1 like-phage Myoviridae can infect the Genus Salmonella (**Bodier-Montagutelli *et al*., 2017**).

The results of TEM φSC4 showed the characteristics belongs to Podoviridae and φSP1 is a classified as Myoviridae. Salmonella lytic phage isolated by **Islam *et al* (2020)**, belongs to the Podoviridae family; **Esmael *et al* (2021)**, belong to the Myoviridae Family (**Samson *et al*., 2013**); and **Li *et al* (2021)**, included in the Siphoviridae family. The three studies above show that Salmonella phages belong to the Order Caudovirales. Most of the Caudovirales were described as lytic phages (96%). Phages belonging to the order Caudovirales have linear and uncoated double-stranded DNA; most of them display contractile, non-contractile or short tails (**Bodier-Montagutelli *et al*., 2017**).

Nucleic acid type test result showed eight bacteriophages from Kencong and three from Puger having DNA nucleic acids. The DNA of the 8 genomes of the isolate was degraded by the DNAse but was not degraded by RNase. DNAse is enzymes that play a role in the process of DNA degradation (**Bodier-Montagutelli *et al*., 2017**). The type of bacteriophage nucleic acid can be determined by comparing the results of genome visualization by applying DNAse and RNAse enzymes. If the bacteriophage genome is degraded by DNAse, it means that the nucleic acid owned by the bacteriophage is DNA, and if the bacteriophage genome is degraded by RNAse, it means that the nucleic acid owned by the bacteriophage is RNA (Bao et al., 2011). Bacteriophage *Salmonella* spp. identified as bacteriophages with DNA nucleic acids have also been reported in various other studies. These include four lytic bacteriophages *Salmonella* isolated from lagoon namely φPR04-1, φPR04-16, φPR21-11, and φPR21-26 (**Counis and Torriglia, 2006**), as well as Bacteriophages PSPu-95 and PSPu-4-116 isolated from SPu-95 and SPu-Salmonella pullorum 116 (**McLaughlin and King, 2008**).

Bands that are thick on the SDS-PAGE gel indicate bands from major proteins and vice versa, bands that are thin on the SDS-PAGE gel indicate bands from minor proteins. Differences that appear when observing protein profiles will result in differences in host range and differences in plaque morphology, even though bacteriophages are from the same family, each bacteriophage has unique structural proteins depending on its morphological type (Ngangbam and Devi, 2012). Minor proteins in bacteriophages are responsible for capsid structure formation and DNA packaging (Drogge et al., 2000), most proteins owned by bacteriophages have a function to help stabilize the structure of the bacteriophage itself. **Kerketta, *et al* (2014)** reported that the *Salmonella*-infecting STIz1 bacteriophage had 2 major proteins measuring 36 kDa and 29 kDa and 2 minor proteins, 59 kDa and 16 kDa, respectively. **Turner *et al* (2012)** also reported that salmonella-infecting bacteriophages have 4 major proteins measuring 14 kDa, 40 kDa, 41 kDa, 79 kDa and have 5 minor proteins measuring 10 kDa, 19 kDa, 20 kDa, 55 kDa, 90 kDa. All the bacteriophages that have been isolated by the researchers belong to the Siphoviridae family.

CONCLUSION

Eleven bacteriophages were isolated from washing-water samples (φSC1, φSC2, φSC3, φSC4, φSC5), food samples (φSM1, φSM2, φSM3), and fish-waste samples (φSUT, φSP1, φSP2). The result showed that φSC3, φSC4, φSC5, and φSM1 have broad infection capabilities to the genera of *Salmonella*, *Escherichia*, and *Staphylococcus*. All isolates were classified as bacteriophages with DNA nucleic acids. The morphological observations using TEM indicated that φSC4 isolates belong to the Podoviridae while φSP1 isolates belong to the Myoviridae. Nucleic acid type test result showed eight bacteriophages from Kencong and three from Puger having DNA nucleic acids. Three bacteriophage from Puger fish auction market succeed determination profiling structural protein. Bacteriophage φSUT has one major protein that is 44 kDa and has minor proteins of 170 kDa, 120 kDa, 64 kDa, 37 kDa. Bacteriophage φSP1 has one major protein measuring 34 kDa and minor proteins measuring 52 kDa and 47 kDa. Bacteriophage φSP2 has one major protein measuring 34 kDa and minor proteins measuring 57 kDa, 48 kDa, 42 kDa.

Acknowledgments: We would like to thank to TEM technicians from the Department of Chemistry, Faculty of Mathematics and Natural Science, Gadjah Mada University.

REFERENCES

Abedon, S.T. (2005). *Bacteriophage Ecology*. Cambridge, UK: Cambridge University Press.

Ackermann, H.W. (2009). Phage or phages. *Bacteriophage*, 1(1).

Acosta, C.J., Galindo, C.M., Ochiai, R,L., Danovaro-Holliday, M.C., Oage, A.L. *et al*. (2017). The role of epidemiology in the introduction of VI polysaccharide typhoid fever vaccines in Asia. *J Health Popul Nutr*, 22, 240-245.

Adams, M.H. (1959). *Bacteriophage ecology group*. New York, NY, USA: Interscience Publishers, Inc.

Ameme, D.K., Abdulai, M., Adjei, E.Y., Afari, E.A., Nyarko, K.M. *et al*. (2016). Foodborne disease outbreak in a resource-limited setting: a tale of missed opportunities and implications for response. *Pan African Medical Journal*, 23, 69. https://doi.org/10.11604/pamj.2016.23.69.7660.

Bai, J., Kim, Y.T., Ryu, S., Lee, J.H. (2016). Biocontrol and rapid detection of foodborne pathogens using bacteriophages and endolysins. *Front. Microbiol*, 7(3), 474-489. https://doi.org/10.3389/fmicb.2016.00474 .

Bao, H., Zhang, H., Wang, R. (2011). *Isolation and characterization of bacteriophages of Salmonella enterica serovar Pullorum*. Poultry Science Association Inc, 1-8. https://doi.org/10.3382/ps.2011-01496.

Bao, H., Zhang, P., Zhang, H., Zhou, Y., Wang, R. (2015). Biocontrol of *Salmonella enteriditis* in foods using bacteriophage. *Viruses*, 7, 4836-485. https://doi.org/10.3390/v7082847.

Bardina, C., Colom, J., Spricigo, D.A., Otero, J., Sánchez-Osuna, M. *et al*. (2016). Genomics of three new bacteriophages useful in the biocontrol of *Salmonella*. *Front. Microbiol*, 7, 545. https://doi.org/10.3389/fmicb.2016.00545.

Bhardwaj, N., Bhardwaj, S.K., Deep, A., Dahiya, S., Kapoor, S. (2015). Lytic Bacteriophages as biocontrol agents of foodborne pathogens. *Asian Journal of Animal and Veterinary Advances*, 10(11), 708-723. https://doi.org/10.3923/AJAVA.2015.708.723.

Bintsis, T. (2017). Foodborne pathogens. *AIMS Microbiology*, 3(3), 529–563. https://doi.org/10.3934/microbiol.2017.3.529.

Bodier-Montagutelli, E., Morello, E., L'Hostis, G., Guillon, A., Dalloneau, E., Respaud, R., Pallaoro, N., Blois, H., Vecellio, L., Gabard, J., & Heuzé-Vourc'h, N. (2017). Inhaled phage therapy: a promising and challenging approach to treat bacterial respiratory infections. *Expert Opinion on Drug Delivery*, 14(8), 959–972. https://doi.org/10.1080/17425247.2017.1252329.

Bondy-Denomy, J., Qian, J.E.R.W., Buckling, D.S.A.G., Davidson, A.R., Maxwell, K.L. (2016). Prophages mediate defense against-phage infection through diverse mechanism. *The ISME Journal*, 10(12), 2854-2866. https://doi.org/10.1038/ismej.2016.79.

Brussow, H. (2005). Phage therapy: the Escherichia coli experience. *Microbiology*, 151, 2133–2140. https://doi.org/10.1099/mic.0.27849-0.

Clokie, M.R.J., Millard, A.D., Letarov, A.V., Heaphy, S. (2011). Phages in nature. *Bacteriophage*, 1(1), 31-45. https://doi.org/10.4161/bact.1.1.14942.

Counis, M.F., Torriglia, A. (2006). Acid dnases and their interest among apoptotic endonucleases. *Biochimie*, 12, 1851-8. https://doi.org/10.1016/j.biochi.2006.07.008.
Diaz-Munoz, S., Koskella, B. (2014). Bacteria–phage interactions in natural environments. *Advances in applied microbiology*, 89, 135–183. https://doi.org/10.1016/b978-0-12-800259-9.00004-4.
Doffkay, Z., Dömötör, D., Kovács, T., Rákhely, R. (2015). Bacteriophage therapy against plant, animal and human pathogens. *Acta Biologica Szegediensis*, 59, 291-302.
Drogge, A., Santos, M. A., Stiege, A. C., Alonso, J. C., Lurz, R., Trautner, T. A. and Tavares, P. (2000). Shape and DNA Packaging Activity of Bacteriophage SPP1 Procapsid: Protein Components and Interactions during Assembly. J Mol Biol, (296), 117–132. https://doi.org/10.1006/jmbi.1999.3450.
Ellis, C.B., Winters, A.R. (1969). Isolation of potential MS2 bacteriophage strains. *Biol. Sci*, 85, 336-345.
Esmael, A., Azab, E., Gobouri, A. A., Nasr-eldin, M. A., Moustafa, M. M. A., Mohamed, S. A., Badr, O. A. M., & Abdelatty, A. M. (2021). Isolation and characterization of two lytic bacteriophages infecting a multi-drug resistant *Salmonella typhimurium* and their efficacy to combat salmonellosis in ready-to-use foods. *Microorganisms*, 9(2), 1–19. https://doi.org/10.3390/microorganisms9020423.
Faruk, S.M.O., Azad, C.A.M.M., Nayeem, U.K. (2014). Isolation of cefixime resistance *Salmonella* from hospitals waste and profiling multi-drug resistance pattern of the selected isolates. *International Research Journal of Biological Sciences*, 3(9), 86-92.
Golkar, Z., Bagasra, O., Pace, D. (2014). Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *The Journal of Infection in Developing Countries*, 8(2), 129–136. https://doi.org/10.3855/jidc.3573.
Gordillo Altamirano, F.L., & Barr, J.J. (2019). Phage therapy in the postantibiotic era. *Clinical Microbiology Reviews*, 32(2). https://doi.org/10.1128/cmr.00066-18.
Grygorcewicz, B., Struk, M., Wasak, A., Nawrotek, P. (2015). Effective Bacteriolysis of shiga toxin-producing *Escherichia coli* O157: H7 caused by specific bacteriophage isolated from pig slurry. Acta Sci. Pol. *Zootechnica*, 14(1), 69–76.
Haq, I.U., Chaudhry, W.N., Akhtar, M.N., Andleeb, S., Qodri, I. (2012). Bacteriophages and their implications on future biotechnology: a review. *Virology Journal*, 9(9). https://doi.org/10.1186/1743-422x-9-9.
Herrera-Sánchez, M. P., Castro-Vargas, R. E., Fandiño-De-Rubio, L. C., Rodríguez-Hernández, R., & Rondón-Barragán, I. S. (2021). Molecular identification of fluoroquinolone resistance in salmonella spp. Isolated from broiler farms and human samples obtained from two regions in Colombia. *Veterinary World*, 14(7), 1767–1773. https://doi.org/10.14202/vetworld.2021.1767-1773.
Hyman, P., Abedon, S.T. (2010). Bacteriophage host range and bacterial resistance. *Advances in Applied Microbiology*, 70, 217-48. https://doi.org/10.1016/s0065-2164(10)70007-1.
Iqbal, M., Narulita, E., Zahra, F., Murdiyah, S. (2020). Effect of phage-antibiotic synergism (PAS) in increasing antibiotic inhibition of bacteria caused of foodborne diseases. *J. Infect Dev Ctries*, 14(55), 488-493. https://doi.org/10.3855/jidc.12094.
Islam, M. S., Hu, Y., Mizan, M. F. R., Yan, T., Nime, I., Zhou, Y., & Li, J. (2020). Characterization of salmonella phage LPST153 that effectively targets most prevalent *Salmonella* serovars. *Microorganisms*, 8(7), 1–18. https://doi.org/10.3390/microorganisms8071089.
Jurczak-Kurek, A., Gasior, T., Nejman-Falenczyk, B., Bloch, S., Dydecka, A. *et al*. (2016). Biodiversity of bacteriophages: morphological and biological properties of a large group of phages isolated from urban sewage. *Scientific Report*, 6, 34338. https://doi.org/10.1038/srep34338.
Kakasis, A., & Panitsa, G. (2019). Bacteriophage therapy as an alternative treatment for human infections. A comprehensive review. *International Journal of Antimicrobial Agents*, 53(1), 16–21. https://doi.org/10.1016/j.ijantimicag.2018.09.004.
Kawasaki, T., Narulita, E., Matsunami, M., Ishikawa, H., Shimizu, M. *et al*. (2016). Genomin diversity of large-plaque-forming podoviruses infecting the phytopathogen Ralstonia solanacearum. *Virology*, 492, 73-81. https://doi.org/10.1016/j.virol.2016.02.011.
Kerketta, P., Agarwal, R. K., Rawat, M. Jain, L., Pavan, K. P., Dhanze, H., Suman K. M. and Kumar, A. (2014). Isolation and Characterization of Lytic Bacteriophage (ϕSTIz1) against Salmonella enterica serovars Typhimurium. *Journal of Pure and Applied Microbiology*, 8(6), 4719-4726.
Ko¨hler, T., Donner, V., van Delden, C. (2010). Lipopolysaccharide as Shield and Receptor for R-Pyocin-Mediated Killing in *Pseudomonas aeruginosa*. *Journal of Bacteriology*, 192(7), 1921-1928. https://doi.org/10.1128/jb.01459-09.
Kropinski, A., Prangishvili, D, Lavigne, R. (2009). The creation of a rational scheme for the nomenclature of viruses of bacteria and archaea. *Environm Microbiol*, 11, 2775–2777. https://doi.org/10.1111/j.1462-2920.2009.01970.x.
Kutter, E., & Sulakvelidze, A. (2005). *Bacteriophages Biology and Applications*. CRC Press.
Kwiatek, M., Parasion, S., & Nakonieczna, A. (2020). Therapeutic bacteriophages as a rescue treatment for drug-resistant infections – an in vivo studies overview. *Journal of Applied Microbiology*, 128(4), 985–1002. https://doi.org/10.1111/jam.14535.
Lamy-Besnier, Q., Chaffringeon, L., Lourenço, M., Payne, R. B., Trinh, J. T., Schwartz, J. A., Sulakvelidze, A., & Debarbieux, L. (2021). Prophylactic Administration of a Bacteriophage Cocktail Is Safe and Effective in Reducing *Salmonella enterica Serovar Typhimurium* Burden in Vivo. *Microbiology Spectrum*, 9(1). https://doi.org/10.1128/spectrum.00497-21.
Legotsky, S.A., Vlasova, K.Y., Priyma, A.D., Shneider, M.M., Pugachev, O.D.V.G. *et al*. (2014). Peptidoglycan degrading activity of the broad-range Salmonella bacteriophage S-394 recombinant endolysin. *Biochimie*, 107, 293-299. https://doi.org/10.1016/j.biochi.2014.09.017.
Leskinen, K., Blasdel, B.G., Lavigne, R., Skurnik, M. (2017). RNA-sequencing reveals the progression of phage-host interactions between Phir1-37 and Yersinia enterocolitica. *Viruses*, 8, 111. https://doi.org/10.3390/v8040111.
Li, J., Li, Y., Ding, Y., Huang, C., Zhang, Y., Wang, J., & Wang, X. (2021). Characterization of a novel Siphoviridae *Salmonella* bacteriophage T156 and its microencapsulation application in food matrix. *Food Research International*, 140, 110004. https://doi.org/10.1016/j.foodres.2020.110004.
Malik, D.J., Sokolov, I.J., Vinner, G.K., Mancuso, F., Cinquerrui, S. *et al*. (2017). Formulation, stabilisation and encapsulation of bacteriophage for phage therapy. Adv. *Colloid Interface Sci*, 249, 100-133. https://doi.org/10.1016/j.cis.2017.05.014.
Mangieri, N., Picozzi, C., Cocuzzi, R., Foshino, R. (2020). Evaluation of a potential bacteriophage cocktail for the control of shiga-toxin producing Escherichia coli in food. *Front. Microbiol*. 11, 1801. https://doi.org/10.3389/fmicb.2020.01801.
McLaughlin, M.R., King, R.A. (2007). Characterization of Salmonella bacteriophages isolated from swine lagoon effluent. *Curr Microbiol*, 56(3), 208-211. https://doi.org/10.1007/s00284-007-9057-9.
Melo, L. D. R., Oliveira, H., Pires, D. P., Dabrowska, K., & Azeredo, J. (2020). Phage therapy efficacy: a review of the last 10 years of preclinical studies. *Critical Reviews in Microbiology*, 46(1), 78–99. https://doi.org/10.1080/1040841x.2020.1729695.
Nadi, Z.R., Salehi, T.Z., Tamai, I.A., Foroushani, A.R., Sillanpaa, M., Dallal, M.M.S. (2020). Evaluation of antibiotic resistance and prevalence of common *Salmonella enterica* serovars isolated from foodborne outbreaks. *Microchemical Journal*, 155, 104660. https://doi.org/10.1016/j.microc.2020.104660.
Narulita, E., Addy, H.S., Kawasaki, T., Fujie, M., Yamada, T. (2016). The involvement of the pilq secretin of type iv pili in phage infection in *Ralstonia solanacearum. Biochemical and Biophysical Research Communications*, 469, 868-872. https://doi.org/10.1016/j.bbrc.2015.12.071.
Narulita, E., Sulistyorini, I., Aji, G.P., Iqbal, M., Murdiyah, S. (2018). Isolation and characterization of bacteriophage in controlling Escherichia coli in Jember area, Indonesia. *Asian Jr. of Microbiol. Biotech. Env. Sci*, 19(2), 81-86.
Ngangbam, A.K., Devi, N.B. (2012). Molecular characterization of *Salmonella* bacteriophages isolated from natural environment and its potential role in phage therapy. *IOSR Journal of Agriculture and Veterinary Science*, 29(1), 33-36.
Ochiai, R.L., Acosta, C.J., Danovaro-Holliday, M.C., Baiqing, D., Bhattacharya, S.K. *et al*. (2008). A study of typhoid fever in five asian countries: disease burden and implications for controls. *Bulletin of the World Health Organization*, 86(4), 260-268. https://doi.org/10.2471/blt.06.039818.
Patra, S. D., Mohakud, N. K., Panda, R. K., Sahu, B. R., & Suar, M. (2021). Prevalence and multidrug resistance in Salmonella enterica Typhimurium: an overview in South East Asia. *World Journal of Microbiology and Biotechnology*, 37(11), 1–17. https://doi.org/10.1007/s11274-021-03146-8.
Putra, B.P., Karuniawati, A. (2012). Bakteriofag. *J Indon Med Assoc* sebagai potensi baru tata laksana infeksi bakteri resisten., 62(3).
Sambrook, J.F., Russell, D.W. (2001). *Molecular cloning: a laboratory manual*. USA: Cold Spring Harbor Laboratory Press.
Samson, J.E., Magadan, A.H., Sabri, M., Moineus, S. (2013). Revenge of the phages: defeating bacterial defense. *Nat Rev Microbial*, 11, 675-687. https://doi.org/10.1038/nrmicro3096.
Santos, F., Moreno-Paz, M., Meseguer, I., Lopez, C., Rossello-Mora, R. *et al*. (2011). Metatranscriptomic analysis of extremely halophilic viral communities. *ISME Journal*, 5, 1621–1633. https://doi.org/10.1038/ismej.2011.34.
Shende, R.K., Hirpurkar, S.D., Sannat, C., Rawat, N., Pandey, V. (2017). Isolation and characterization of bacteriophages with lytic activity against common bacterial pathogens. *Vet World*, 10(8), 973-978. https://doi.org/10.14202/vetworld.2017.973-978.
Sillankorva, S.M., Oliviera, H., Azerado, J. (2012). Bacteriophages and their role in food safety. *International Journal Microbiol*, 10: 863-945. https://doi.org/10.1155/2012/863945.
Singla, S., Harjai, K., Katare, O.P., Chhibber, S. (2016). Encapsulation of bacteriophage in liposome accentuates its entry into macrophage and shields it from neutralizing antibodies. *PLoS One*, 11(4), e0153777. https://doi.org/10.1371/journal.pone.0153777.
Skurnik, M., Eckhard, S. (2006). Phage therapy: facts and fiction. *International Journal of Medical Microbiology*, 296, 5–146. https://doi.org/10.1016/j.ijmm.2005.09.002.
Susianto, G., Farid, M.M., Dhany, N.R., Addy, H.S. (2014). Host Range for Bacteriophages that Infect Bacterial Blight Pathogen on Soybean. *Procedia Environmental Sciences*, 20, 760– 766.

Taj, M.K., Samreen, Z., Taj, I., Hassani, T.M., Ling, J.X., Yunlin. (2014). T4 bacteriophage as a model organism. IMPACT: *International Journal of Research in Applied, Natural and Social Sciences*, 2, 2347-4580.

Tarabees, R., Elsayed, M.S.A., Shawish, R., Basiouni, S., Shehata, A.A. (2016). Isolation and characterization of Salmonella Enteritidis and Salmonella Typhimurium from chicken meat in Egypt. *J. Infect Dev Ctries*, 1(4): 3855. https://doi.org/10.3855/jidc.8043.

Wain, J., Simpson, J. A., Thi Diem Nga, L., Song Diep, T., Thanh Duy, P., Baker, S., Day, N. P. J., White, N. J., & Parry, C. M. (2021). Bactericidal activities and post-antibiotic effects of ofloxacin and ceftriaxone against drug-resistant Salmonella enterica serovar Typhi. *Journal of Antimicrobial Chemotherapy*, 76(10), 2606–2609. https://doi.org/10.1093/jac/dkab215.

Weinbauer, M.G. (2004). Ecology of prokaryotic viruses. *FEMS Microbiol Rev*, 28, 127. https://doi.org/10.1016/j.femsre.2003.08.001.

Yamada, T., Kawasaki, T., Nagata, S., Fujiwara, A., Usami, S., Fujie, M. (2007). New bacteriophages that infect the phytopathogen *Ralstonia solanacearum. Microbiology*, 153, 2630–2639. https://doi.org/10.1099/mic.0.2006/001453-0.

Yang, Y., Le, S., Shen, W,, Chen, Q., Huang, Y. *et al*. (2013). Antibacterial activity of a lytic enzyme encoded by Pseudomonas aeruginosa double stranded RNA bacteriophage phiYY. *Frontiers in Microbiology*, 9, 6088179. https://doi.org/10.3389/fmicb.2018.01778.