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Genetic development of Stx phage

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Abstract. *Sjahriani T, Kurniati M, Arivo D, Syukri DM, Tyasningsih W. 2023. Genetic development of Stx phage. Biodiversitas 24: 4334-4341.* Stx phages have been studied and described due to their involvement in shigatoxin. The variety, evolution, dispersion, and molecular mechanisms of STEC and Stx phages have all been widely studied, but further research is still required. The objective of this study was to investigate the unique sequences of bacteriophage DNA from the NCBI database and to identify the changes in the DNA sequence of both regular and Stx-carrying bacteriophages. It was first reported in 2001, but studies have not been carried out. The Stx-carrying bacteriophage DNA sequence, similarity of identity, e-value, and gaps were analyzed using PCR and DNA sequencing. The number of bacteriophage used in this study was eight. The results revealed the similarity of bacteriophage to Stx-carrying bacteriophages by 82.67% to 88.5%, and exhibited notable variations in comparison to each phage 1 to 8 (*Eco*157 *phage* WASJ/2022) ($p<0.001$). Moreover, results exhibited an e-value ranging from 7e-85 to 3e-44. In addition, results showed gaps between 1-2% in comparison to other Stx-carrying bacteriophages in NCBI with bacteriophage alterations on the bases of Cytosine and Thymine in 184th and 263rd. Therefore, this work provided the potency of bacteriophage as an alternative pharmaceutical in controlling foodborne diseases.

Keywords: Foodborne disease, genetic, phage, STEC, Stx

INTRODUCTION

In 2019, the largest contribution of cattle in Indonesia was in East Java, there were approximately 4.705.067 (27.79%) of cattle (Central Statistics Agency of Indonesia 2019a). East Java produces around 21.95% of its meat from cattle slaughtered, and the majority of them are consumed by local people (Central Statistics Agency of Indonesia, 2019b). The disposal from abattoir causes a pollution in the Kalimas River due to the dysfunction of wastewater treatment. Fundamentally, the food industry in East Java has employed a range of sanitation procedures to improve the fresh produce's microbiological safety and quality (Fisher et al. 2016; Joshi et al. 2018). The government of East Java has been working to raise the Human Development Index and reduce the poverty rate. However, the percentage of people in poverty in East Java is higher by 10.86% compared with the national average of poverty rate, particularly in rural areas (Sjahriani et al. 2021a).

In Indonesia, the prevalence of food poisoning cases has reached by 80.83% (National Agency of Drug and Food Control of Indonesia 2016). Foodborne diseases and food poisoning have gained attention of medical community, particularly in developing countries (Carbas et al. 2013). It is estimated that *Escherichia coli* O157:H7 causes 63,000 cases of foodborne infections, 2,100 hospitalizations, 20 fatalities, and high cost of $271 million (Hoffmann et al. 2015; Poxleitner et al. 2016). *Escherichia coli* O157:H7 can produce shigatoxin that is suspected to alter the type and extent the impairment of human tissue, leading to catastrophic renal failure, bloody diarrhea, and death (Melton-Celsa 2014; Luna-Gierke et al. 2014). Therefore, the food industries use a variety of sanitation techniques to improve the microbiological safety and quality of fresh food.

Bacteriophages get attached to the cells surfaces of bacteria through certain surface receptors of host cell (Zhan et al. 2015). Phage is believed to extract and infect *E. coli* serotypes or other members of the *Enterobacteria*ceae family (Khalil et al. 2016). Furthermore, the genetic material of bacteriophage is injected into the cytoplasm of the bacterial cell, resulting an infection (Silva et al. 2016). Bacteriophages provide a safe and natural way to prevent the spread of pathogen in humans, due to their natural component (Bhardwaj et al. 2015). It is primarily composed a non-harmful nucleic acid and a member of the human microbiome (Huh et al. 2019). The infection of bacteriophage is initiated by perforation of bacterial cell wall, and viral DNA is transferred into the cytoplasm of host cells (Fokine and Rossmann 2014). The Rz/Rz1 protein involved in the outer membrane breakdown, resulting in bacterial lysis (Catalao et al. 2013). The analysis of DNA phage sequences offers an alternative treatment of foodborne infections due to the growing incidence of antibiotics resistance (Aslam et al. 2018). This work provided a profitable, prices and targeted approach since the variety, evolution, dissemination, and molecular mechanisms of STEC and Stx phages have been studied in

2001. However, further study is still required in these areas, whereby the changes of Stx-carrying bacteriophage's DNA sequence have never been accomplished previously. In general, this study analyzed the unique DNA sequences of Stx-carrying bacteriophages, followed with the founding of nucleotide variations in their DNA sequences, compared to bacteriophage DNA sequences in the NCBI database.

MATERIALS AND METHODS

Study area

The samples were collected using purposive sampling, from the reservoirs of wastewater. The samples were collected from Surabaya, East Java of Indonesia (longitude of 7º13'13.2" S to 7º18'06.6" S and latitude of 112º44'13.6"S to 112º44'44.3") (Sjahriani et al. 2021a) (Figure 1, Table 1).

The preparation of bacteriophage

Escherichia coli O157:H7 bacteriophage isolates were obtained from the environmental sewage from Surabaya, Indonesia and kept in laboratory Department of Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia (Sjahriani et al. 2021a). In brief, 15 mL of sample was taken using a sterile tube. Subsequently, 1 mL of liquid waste sample was diluted into 9 mL of Nutrient Broth (Oxoid, UK), centrifuged at 3000 rpm for 20 min. Furthermore, the supernatant was filtered using 0.45 µm millipore membrane (Minisart, Sartorius). A 4.5 mL filtrate was mixed with 0.5 mL of *E. coli* O157:H7 that were adjusted to 1.5×10^8 CFU/mL, followed by adding 5 mL of Nutrient Broth. Afterwards, the mixture was incubated for 24 hours in a water bath at 37°C. It was then centrifuged at 3000 rpm at 4°C for 15 min. The supernatant was taken by a syringe and filtered using 0.22 µm millipore membrane (Minisart, Sartorius). The supernatant was stored into a sterile tube at low temperature (Thung et al. 2017).

Extraction and amplification of bacteriophage DNA target

The bacteriophage DNA extraction used Qiagen technique. In brief, each bacteriophage culture was centrifuged for 3 min at 13,000 rpm. Subsequently 5 µL template was placed in a 12.5 µL volume of 2x Intron master mix. The PCR mixture consisted of 0.5 µL distilled water, 1 µL of forward primer, and 1 µL of reverse primer. The thermal cycler gene was conducted using PCR (BioRad, Tokyo, Japan). Furthermore, the cycle program of denaturation, annealing, and extension temperatures was: 1 cycle at 94°C for 5 min and 35 cycles at 94°C for 45 sec, 30 sec at 59°C, and 30 sec at 72°C, with the last extension at 72°C for 5 min (Qiagen, Maryland, USA).

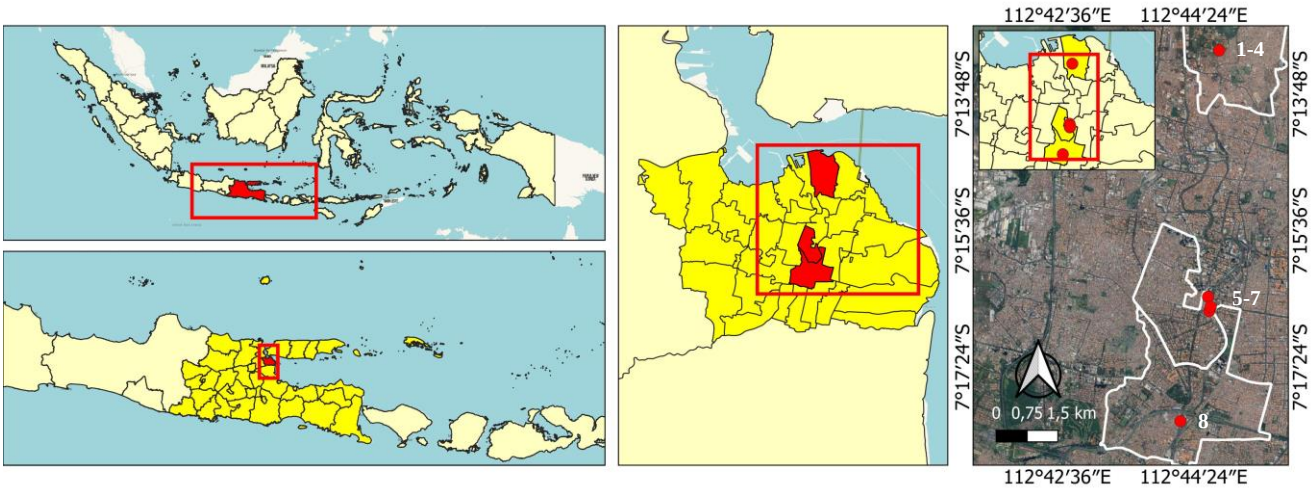

Figure 1. Location of sample in Surabaya, East Java of Indonesia (see Table 1)

Table 1. Collection of bacteriophage samples

No.	Bacteriophage samples	Longitude	Latitude
1	Cow sewage from abattoir	7°13'13.2"S	112°44'44.3"E
2	Goat sewage from abattoir	7°13'13.2"S	112°44'44.3"E
3	Pig sewage from abattoir	7°13'13.2"S	112°44'44.3"E
4	Abattoir drainage sewage	7°13'13.2"S	112°44'44.3"E
5	Chicken sewage from traditional market	7°16'39.5"S	112°44'36.4"E
6	Fish sewage from traditional market	7°16'28.2"S	112°44'35.4"E
7	Kalimas river sewage	7°16'36.0"S	112°44'37.6"E
8	Cow sewage from traditional market	7°18'06.6"S	112°44'13.6"E

Agarose gel electrophoresis

The size of the DNA product was estimated using a 100-bp DNA ladder marker (NEXMark). A 4 µL sample of each PCR product was electrophoresed on a 2% agarose gel at 100 volts for 30 min. The developing bands were visualized using a UV transilluminator (Sjahriani et al. 2021b).

Purification and sequencing of DNA bacteriophage

Purification and sequencing of DNA were conducted according to previous report (Thung et al. 2017; Sjahriani et al. 2021b). They were demonstrated using Thermo Fisher Scientific, California, USA. Moreover, DNA sequencing was performed by division of Malaysia's 1st Base DNA Sequencing. Furthermore, the sequencing results were evaluated using NCBI BLAST.

Data analysis

The Kruskal-Wallis statistical analysis were conducted on this study.

RESULTS AND DISCUSSION

Detection of bacteriophage gene

Previous study has showed the findings of molecular identification using the PCR technique (Sjahriani et al. 2021b).

The Bacteriophage of 1 to 8 (*Eco*157 WASJ/2022) genes were visualized in a 300 bp fragment of DNA. PCR results in the eight potential bands of gene were applied to confirm the DNA sequencing. Subsequently, results were compared with GenBank sequence data of NCBI. The NCBI Blast tool was required, to match the sequencing findings (Table 2).

Bacteriophage DNA sequence

DNA sequence of bacteriophage was developed by utilizing the results of DNA sequence of bacteriophage 1, 2, 3, 4, 5, 6, 7, and 8 (Figure 2). According to previous study that bacteriophage of 1 to 8 showed a different DNA sequence compared with NCBI database, it might be due to the role of habitat and environmental factors, including temperature and pH (Denes and Wiedmann 2014; Ly-Chatain 2014; Thung et al. 2017).

DNA sequences similarity of bacteriophage

The NCBI Blast program was demonstrated to analyze the similarity of DNA sequence of bacteriophage. Results are presented in Table 2.

The similarity of DNA sequence of bacteriophage results exhibited range of 82.67 to 88.56%. Moreover, DNA sequence of bacteriophage showed e-values and gaps range of 7e-85 to 3e-44 and 1-2%, respectively (Table 2). The results of DNA phage revealed the differences of similarity if compared with the DNA of bacteriophage from NCBI database, including DNA of verotoxin-carrying *Enterobacteria* phage, *Shigella* phage, Stx phage, *Escherichia* Stx1 phage, Stx1 phage, Stx2 phage, and Stx2a phage. As can be seen from the bacteriophage DNA sequences provided above, each Stx-carrying bacteriophage has a distinct DNA sequence. By comparing homologous sequences that retrieved from NCBI BLAST, discovered that bacteriophage of 1 to 8 (*Eco*157 *phage* WASJ/2022) and the Stx-carrying phage (Table 2) shared roughly 274 base pairs. Further analysis showed that the homology of *Enterobacteria* phage VT-2 and prophage inserted region in *E. coli* O157:H7. It was found in regions 27907 to 28181, while the homology of *Escherichia* Stx1 converting recombinant phage HUN/2013 was found in regions 50241 to 50515. Moreover, *Shigella* phage 75/02 Stx, Stx1 converting phage, Stx2 converting phage 1, Stx1 converting phage, Stx2 converting phage I, Stx2 converting phage II, Stx2 converting phage vB_EcoP_24B, Stx2 converting phage Stx2a_F403 proviral, Stx2 converting phage Stx2a_F422 proviral, Stx2 converting phage Stx2a_F451 proviral, Stx converting phage vB_EcoS_P22, Stx converting phage vB_EcoS_P32, Stx converting phage vB_EcoS_P27, Stx2-converting phage Stx2a_WGPS9 proviral, Stx2-converting phage Stx2a_F765 proviral, Stx2-converting phage Stx2a_F723 proviral, Stx2a-converting phage Stx2_EH2011, Stx2a-converting phage Stx2_16002, Stx2a-converting phage Stx2_09E025, Stx2-converting phage 86, Stx1 converting phage AU6Stx1, and Stx1 converting phage AU5Stx1 in regions 59534 to 59808, 60475 to 60713, 27907 to 28181, 29481 to 29755, 29095 to 29369, 35091 to 35365, 28180 to 28454, 28189 to 28463, 28895 to 29169, 22243 to 22517, 32453 to 32727, 31118 to 31392, 32245 to 32519, 29212 to 29486, 32241 to 32515, 6991 to 7265, 27375 to 27649, and 28623 to 28897, respectively. Additionally, the Bioedit application was conducted to examine the number of bacteriophages bases that changed between 184th and 263rd of DNA sequences, shown in Figure 3.

The Bacteriophage of 1 to 8 (*Eco*157 WASJ/2022) visualized genes were applied to confirm the DNA sequencing, and the results were compared with NCBI GenBank sequence data using the NCBI Blast tool.

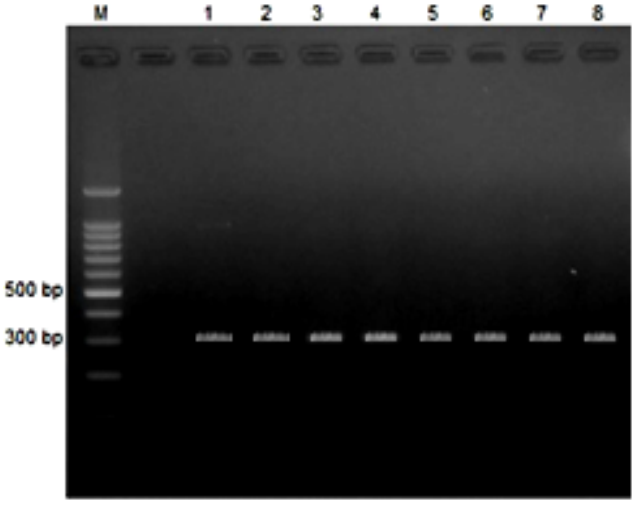

Figure 2. Observed band of phage 1 to phage 8 gene (M = Marker)

Table 2. DNA sequence similarity of bacteriophage

Phage	Description	Phage 1	Phage 2	Phage 3	Phage 4	Phage 5	Phage 6	Phage 7	Phage 8	NCBI acc. no.
Enterobacteria phage VT-2 genomic DNA, prophage inserted region in *Escherichia coli* O157:H7	Similarity (%)	88.41	87.82	88.32	88.00	87.59	88.32	88.32	88.56	AP000422.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Escherichia Stx1 converting recombinant phage HUN/2013, complete genome	Similarity (%)	88.41	87.82	88.23	88	87.59	88.32	88.32	88.56	KJ909655.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Shigella phage 75/02 Stx, complete genome	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	KF766125.2
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx1 converting phage DNA, complete genome	Similarity (%)	88.41	87.82	88.32	88.00	87.59	88.32	88.32	88.56	AP005153.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Stx2 converting phage I DNA, complete genome	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	AP004402.1
	E-value	6e-86	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2 converting phage II DNA, complete genome	Similarity (%)	88.41	87.82	88.32	88	87.59	88.32	88.32	88.56	AP005154.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Stx2 converting phage vB_EcoP_24B, complete genome	Similarity (%)	88.41	87.82	88.32	88	87.59	88.32	88.32	88.56	HM208303.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Stx2 converting phage Stx2a_F403 proviral DNA, complete genome	Similarity (%)	88.41	87.82	88.32	88	87.59	88.32	88.32	88.56	AP012529.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Stx2 converting phage Stx2a_F422 proviral DNA, complete genome	Similarity (%)	88.41	87.82	88.32	88	87.59	88.32	88.32	88.56	AP012531.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Stx2 converting phage Stx2a_F451 proviral DNA, complete genome	Similarity (%)	88.41	87.82	88.32	88	87.59	88.32	88.32	88.56	NC_049924.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Stx converting phage vB_EcoS_P22, complete genome	Similarity (%)	88.41	87.82	88.32	88	87.59	88.32	88.32	88.56	KU238069.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Stx converting phage vB_EcoS_P32, complete genome	Similarity (%)	88.41	87.82	88.32	88	87.59	88.32	88.32	88.56	KU238068.1
	E-value	6e-86	2e-81	7e-85	9e-84	3e-78	7e-85	7e-85	7e-85	
	Gaps (%)	1	1	1	1	2	1	1	1	
Stx converting phage vB_EcoS_ST2-8624, complete genome	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	NC_049922.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	

Stx converting phage vB_EcoS_P27, complete genome	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	NC_049925.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2-converting phage Stx2a_WGPS9 proviral DNA, complete genome	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	NC_049923.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2-converting phage Stx2a_F765 proviral DNA, complete genome	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	AP012534.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2-converting phage Stx2a_F723 proviral DNA, complete genome	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	AP012533.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2a-converting phage Stx2_EH2011 DNA, complete sequence	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	LC645443.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2a-converting phage Stx2_16002 DNA, complete sequence	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	LC645439.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2a-converting phage Stx2_09E025 DNA, complete sequence	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	LC645438.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2-converting phage 86 DNA, complete sequence	Similarity (%)	86.23	86.25	86.13	86.55	85.71	86.13	86.13	86.35	AB255436.1
	E-value	6e-76	1e-73	7e-75	4e-77	7e-70	7e-75	7e-75	7e-75	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx1 converting phage AU6Stx1, complete genome	Similarity (%)	85.87	85.87	85.77	86.18	85.34	85.77	85.77	85.98	NC_049921.1
	E-value	3e-74	4e-72	3e-73	2e-75	3e-68	3e-73	3e-73	3e-73	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx1 converting phage AU5Stx1, complete genome	Similarity (%)	85.87	85.87	85.77	86.18	85.34	85.77	85.77	85.98	NC_049920.1
	E-value	3e-74	4e-72	3e-73	2e-75	3e-68	3e-73	3e-73	3e-73	
	Gaps (%)	1	2	1	1	2	1	1	1	
Stx2d-converting phage Stx2_112808 DNA, complete genome	Similarity (%)	83.26	82.43	83.11	-	83.49	83.11	83.11	83.33	LC567830.1
	E-value	1e-48	3e-44	1e-47		6e-46	1e-47	1e-47	1e-47	
	Gaps (%)	2	2	2		1	2	2	2	
Stx2a-converting phage Stx2_499 DNA, complete genome	Similarity (%)	82.82	82.73	82.67	-	83.10	82.67	82.67	-	LC567824.1
	E-value	5e-47	8e-45	6e-46		8e-45	6e-46	6e-46		
	Gaps (%)	2	2	2		1	2	2		

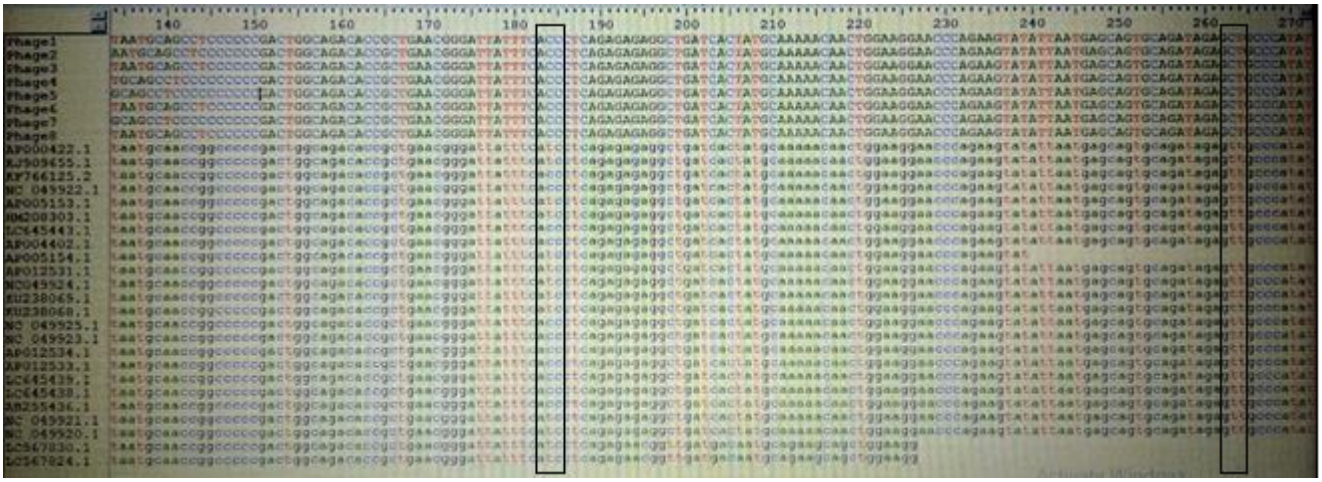

Figure 3. Cytocin and thymine changes in the DNA sequence

On the contrary, *Enterobacteria* phage VT-2 and prophage inserted region of *E. coli* O157:H7, *E.* Stx1 converting recombinant phage HUN/2013, Stx1 converting phage, Stx2 converting phage II, Stx2 converting phage vB_EcoP_24B, Stx2 converting phage Stx2a_F403 proviral, Stx2 converting phage Stx2a_F422 proviral, Stx2 converting phage Stx2a_F451 proviral, Stx converting phage vB_EcoS_P22, Stx converting phage vB_EcoS_P32, Stx1 converting phage AU6Stx1, Stx1 converting phage AU5Stx1, which carries the thymine base in that position. Bacteriophage 1 through Bacteriophage 8 carry the cytosine base in the 184th DNA sequence. Additionally, it was observed that, in contrast to *Enterobacteria* phage VT-2, prophage inserted region in *E. coli* O157:H7, *Escherichia* Stx1 converting recombinant phage HUN/2013, *Shigella* phage 75/02 Stx, Stx1 converting phage, Stx2 converting phage I, Stx2 converting phage II, Stx2 converting phage vB_EcoP_24B, Stx2 converting phage Stx2a_F422 proviral, Stx2 converting phage Stx2a_F451 proviral, Stx converting phage vB_EcoS_P22, Stx converting phage vB_EcoS_P32, Stx converting phage vB_EcoS_P27, Stx2-converting phage Stx2a_WGPS9 proviral, Stx2-converting phage Stx2a_F765 proviral, Stx2-converting phage Stx2a_F723 proviral, Stx2a-converting phage Stx2_EH2011, Stx2a-converting phage Stx2_16002, Stx2a-converting phage Stx2_09E025, Stx2-converting phage 86, Stx1 converting phage AU6Stx1, Stx1 converting phage AU5Stx1 Stx1 converting phage AU5Stx1, furthermore, Bacteriophage 1 through Bacteriophage 8 have cytosine bases in the 263rd DNA sequence (T→C).

Stx phage integration locations in entire genomes were widely varied, even among phages encoding the same Stx subtype (Nakamura et al. 2020). Meanwhile, there have been fewer reports of inducible or transducible bacteriophages carrying Stx1 compared to the numerous inducible and transducible Stx2-containing phages (Sváb et al. 2015). However, a previous study reported a transferable Stx1 converting phage from a *Shigella sonnei* clinical strain (Nógrády et al. 2013). It carried the genomic structure of the Stx1 phage as well as other Stx phages. When compared to the StxB gene in *S. dysenteriae* type 1, the Stx genes carried by *S. sonnei* 75/02 and 7888 (AJ279086) strains share the same synonymous SNP (C→T, location 22,084 in *Shigella* phage 75/02 Stx). The phage *Shigella* 75/02 Stx remained inducible and persistent in its bacterial hosts. The site was chosen since it was the same area where the Stx1 converting phage POCJ13 was discovered in *S. flexneri* strains (Gray et al. 2014). *Shigella* phage 75/02 Stx's overall genomic arrangement was found to be circular. Intriguingly, the comparative genome analysis identified a significant region in *Shigella* Stx1-converting phages that is co-linear with multiple Stx2 prophages, especially those carried by EHEC O157:H7 strains, indicating a shared origin for the relevant regions. These characteristics point to a recombination between a phage carrying Stx1 and one carrying Stx2, during which the area containing the Stx2 genes was swapped out for one having the Stx1 genes. This type of recombination has commonly been observed for inducible Stx1 phages in *E coli* strains that have both toxin types, even though the parental strain only had the Stx1 gene cluster (Sváb et al. 2015), despite the parental strain only had the Stx1 gene cluster.

Previous study showed the variability of virulence among O157 strains and that phage diversity can influence the production of virulence components expressed by phages (Lennon et al. 2020). Given their shorter generation cycles and ease of recombination with other phages, phages exhibit a very high level of genetic diversity, as shown by the widespread genomic mosaicism found in phage genomes. These phage mutations could have an impact either directly or indirectly. Stx phages have been extensively characterized due to the position of EHEC strains as major pathogens. Moreover, the role they play in the dispersion of their primary virulence components, the Shiga toxins. The existence of the lambda-red recombinase system encoded by Stx phages, as well as other factors, have been linked to the likelihood of new phages emerging from STEC strains by recombination (De Paepe et al.

2014). The study demonstrated that recombination of Sakai phages occurs, and Recombination can be a crucial factor in the evolution of Stx phages, as it is probable that comparable recombinant and transducible phages will emerge in the wild, contributing to the widespread dispersal of Stx genes (Ogura et al. 2015). Previous study has shown a significant correlation between the Stx2a phage subtype and the level of Stx2 production in O157 strains, as evidenced by subtyping and Stx2 production level analysis. The Stx2a phage subtype may be one of the crucial factors to determine it, and the Stx2a production levels of each O157 strain appear to be predictable by subtyping their Stx2a phages. Other factors, such as genetic backgrounds associated with the lineages of host strains, could also affect the level of Stx2 production by O157. The ability to produce the highest level of Stx2 was conferred by the Stx2a_ subtype, and host strains carrying this subtype produced more Stx2 than strains carrying the other two subtypes of Stx2. It is believed that the phage's late gene promoter's effectiveness in inducing the phage is critical in determining the degree of Stx2 expression (Ogura et al. 2015). Stx phage CP-933V (AE005174) in Stx1 and Stx phage BP-933W (AE005174) in Stx2 were also declared by studying the sequence of phage DNA. Therefore, it can be used as an alternative of pharmaceuticals in the treatment of foodborne diseases.

In conclusion, overall, by comparing homologous sequences, it was found that each Stx-carrying bacteriophage, including *Enterobacteria* phage VT-2, *Escherichia* Stx1, *Shigella* phage 75/02 Stx, Stx1 converting phage, Stx2 converting phage 1, Stx1 converting phage, Stx2 converting phage I, Stx2 converting phage II, Stx2 converting phage vB_EcoP_24B, Stx2 converting phage Stx2a_F403 proviral, Stx2 converting phage Stx2a_F422 proviral, Stx2 converting phage Stx2a_F451 proviral, Stx converting phage vB_EcoS_P22, Stx converting phage vB_EcoS_P32, Stx converting phage vB_EcoS_P27, Stx2-converting phage Stx2a_WGPS9 proviral, Stx2-converting phage Stx2a_F765 proviral, Stx2-converting phage Stx2a_F723 proviral, Stx2a-converting phage Stx2_EH2011, Stx2a-converting phage Stx2_16002, Stx2a-converting phage Stx2_09E025, Stx2-converting phage 86, Stx1 converting phage AU6Stx1, Stx1 converting phage AU5Stx1 and the Bacteriophages 1 to 8 shared around 274 base pairs, which varied between the 184th and 263rd position of Cytocin and Thymine in the DNA sequences. Thus, this study could be as an alternative strategy in order to determine the appropriate intervention to control foodborne diseases.

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