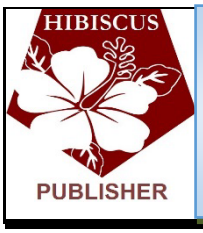
JOBIMB, 2025, Vol 13, No 1, 42-47
https://doi.org/10.54987/jobimb.v13i1.1074



JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY
Website: http://journal.hibiscuspublisher.com/index.php/JOBIMB/index

Characterisation of *Salmonella enterica* serovar Typhimurium Bacteriophage vB_SenS_ST10: Host-Range Determination, Efficiency of Plating, and Antibiofilm Activity

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HISTORY

Received: 19th Jan 2025
Received in revised form: 21st March 2025
Accepted: 10th April 2025

KEYWORDS

Antimicrobial resistance
Poultry farming
Bacterial control
Viral therapy
Food safety

ABSTRACT

The Malaysian poultry industry, a significant contributor to the agricultural sector and national GDP, is increasingly challenged by antibiotic-resistant bacterial infections, particularly from *Salmonella enterica* serovar Typhimurium. The widespread use of antibiotics in poultry farming has driven the emergence of resistant strains, highlighting the need for alternative antimicrobial strategies. This study evaluated the characteristics and therapeutic potential of bacteriophage vB_SenS_ST10 as a biological control agent against *Salmonella* infections in poultry. Host range analysis revealed that vB_SenS_ST10 exhibited a narrow host range, effectively infecting only three of the thirty-two bacterial isolates tested: *S.* Typhimurium 8720/06, *S. enterica* (SCC), and *S.* Tennessee. Efficiency of plating (EOP) analysis indicated reduced binding efficiency for *S. enterica* (SCC) with an EOP value of 1.5 x 10^{-2} relative to the reference strain. Biofilm inhibition assays demonstrated significant ($P < 0.05$) biofilm suppression at phage concentrations above 10^4 PFU/mL, though a plateau was observed at higher levels, and complete biofilm eradication was not significantly achieved even at 10^9 PFU/mL. Importantly, vB_SenS_ST10 did not affect beneficial gut bacteria, such as *Lactobacillus* and *Bifidobacterium* species, supporting its potential for targeted antimicrobial application without disrupting gut microbiota. The selective nature of vB_SenS_ST10, combined with its ability to inhibit biofilm formation, presents a promising approach to mitigate *Salmonella*-associated contamination in poultry production. However, further research is necessary to optimise its application and investigate mechanisms underlying biofilm resilience in poultry production systems.

INTRODUCTION

The poultry industry represents a significant component of Malaysia's food industry and agricultural landscape. Malaysia ranks among the top global consumers of poultry meat, with per capita consumption reaching an estimated 50 kilograms in 2023 [1]. This high consumption rate reflects poultry's position as a popular and affordable protein source, with demand driven by population growth, rising income levels, and urbanization. The substantial growth in consumption and the industry's intensive farming practices have created environments that can facilitate bacterial pathogen proliferation, particularly *Salmonella* species. *Salmonella enterica* serovar Typhimurium has emerged as a critical concern in poultry production, posing significant risks to both animal health and food safety [2]. These Gram-negative bacteria can persist in various environments, forming resilient biofilms that contribute to their survival in poultry farms and processing facilities [3]. The bacteria's ability to establish biofilms in the gut and hepatobiliary system of broilers leads to acute, latent, or chronic disease manifestations, while also enhancing their long-term persistence through the production of an extracellular polymeric matrix substances (EPS) [4, 5].

Recent studies indicate an alarming trend in antimicrobial resistance among *Salmonella* strains globally and in Malaysia. Studies from Malaysian poultry operations have revealed concerning resistance patterns, with predominant resistance to sulfonamides (52%), tetracycline (39.5%), and aminoglycosides (35.6%) [6]. In comparison, studies from Italy have shown that 80% of isolated *Salmonella* strains exhibit multi-drug resistance

(MDR), with particularly high resistance (72.5%) to tetracyclines [7]. This rising trend in antimicrobial resistance poses a significant threat to both poultry production and public health, particularly as these resistant strains can be transmitted to humans through the consumption of infected chicken meat. Bacteriophage therapy has emerged as a promising alternative to traditional antibiotic treatments. These viruses specifically target and kill bacteria, offering several advantages over conventional antimicrobial treatments, including high host specificity, self-limiting amplification capabilities, and minimal impact on beneficial gut microbiota [8, 9]. The natural occurrence of bacteriophages in environmental systems also makes them an environmentally sustainable option for bacterial control [10].

Recent studies have demonstrated compelling evidence for the effectiveness of bacteriophage therapy in controlling *Salmonella* Typhimurium infections in chickens. Chicken trials have shown that phage treatment can significantly reduce mortality rates from 51.1% in untreated infected controls to just 11.1% in birds treated with combined phages [11]. Quantitative assessments have revealed that phage therapy can achieve substantial bacterial load reductions, with *S.* Typhimurium concentrations decreasing from ~6.75 $\log_{10}$ CFU/g in untreated birds to ~2.73 $\log_{10}$ CFU/g in phage-treated birds within 24 h post-treatment [12]. The efficacy of phage therapy matches that of conventional antibiotics while offering improved safety, as phage treatment avoids the adverse effects on blood parameters and liver function typically seen with antibiotics like enrofloxacin and colistin [12].

In addition to reducing bacterial load, studies show that phage cocktails can disrupt biofilm formation, with some achieving up to 74.26% biofilm removal in laboratory settings [13]. However, fully eradicating biofilms remains challenging due to the protective extracellular polymeric substance (EPS) matrix that shields bacterial cells within biofilms, restricting phage access and reducing treatment effectiveness [14, 15]. Additionally, bacteria in biofilms often enter a dormant state, making them less susceptible to phage-mediated lysis [16]. This persistence of biofilm-associated bacteria is particularly concerning in poultry production, where biofilms on equipment and surfaces serve as reservoirs for pathogens and antibiotic-resistant strains. Consequently, research focusing on phage efficacy in biofilm environments is crucial, as it addresses a major barrier to effective biocontrol, offering insights into more sustainable solutions for managing resistant bacterial infections in the food industry.

Building on these findings, this study investigates the characteristics of *Salmonella enterica* serovar Typhimurium bacteriophage vB_SenS_ST10 , focusing on its host range, efficiency of plating (EOP), and biofilm control capabilities. Understanding these properties is crucial for evaluating vB_SenS_ST10's potential as a biocontrol agent in the poultry industry. The research aims to contribute to the growing body of knowledge on phage-based alternatives to conventional antibiotics, addressing the urgent need for sustainable solutions to combat antimicrobial resistance in the poultry industry.

MATERIALS AND METHODS

Preparation of Media and Buffers

For bacterial culture cultivation, various media were employed: Brain Heart Infusion (BHI) agar (1.5%) from Condalab (Spain) and Luria-Bertani (LB) agar (1.5%) from Sigma-Aldrich (US) were used as general media. Selective media included Xylose-Lysine Deoxycholate (XLD) agar (Oxoid, England), MacConkey agar (Merck, Germany), Mannitol Salt agar (Merck, Germany), Azide Dextrose agar, Proteae Selective agar, Listeria Selective agar, and Charcoal Cefoperazone Deoxycholate (CCDA) agar (Merck, Germany). All media were prepared according to the manufacturers' instructions, autoclaved at 121°C for 15 minutes, and stored at 4 °C. CCDA agar was supplemented with CCDA Selective Supplement (Merck) at 2 mL per 500 mL to maintain its selectivity. Buffers used included Sodium-Magnesium (SM) buffer and Phosphate Buffered Saline (PBS). SM buffer was prepared by dissolving 2.9 g NaCl, 1 g $MgSO4 \cdot 7H_2O$, 1 g Tris-HCl (Vivantis, Malaysia), and 0.01 g gelatin (Nacalai Tesque, Japan) in 500 mL distilled water and adjusted to pH 7.4. PBS buffer comprised 4 g NaCl, 0.1 g KCl (R&M Chemicals, Malaysia), 0.72 g sodium phosphate dibasic (Bio Basic Inc., Canada), and 0.12 g potassium phosphate monobasic (Sigma-Aldrich, US) in 500 mL distilled water, adjusted to pH 7.4. Both buffers were autoclaved at 121 °C for 15 minutes.

Revival of Glycerol Stock Cultures

Glycerol stocks of various *Salmonella enterica* serovars were obtained from the Food and Microbiome Technology Laboratory (FAMTECH), Universiti Putra Malaysia, for experimental use. These stocks were revived through standard aerobic and anaerobic subculturing techniques. Anaerobic culturing was performed for *Campylobacter jejuni* and *Bifidobacterium* species using nitrogen-flushed bags incubated at 37 °C.

ST10 Phage Viability and Titre Determination

ST10 phage stock, provided by FAMTECH Laboratory, was thawed and assessed for viability through a double-agar overlay plaque assay using the *S.* Typhimurium 8720/06 host. Briefly, phage lysates and host cultures were combined in molten soft agar and spread over BHI plates. Plates were incubated overnight at 37 °C, with plaque formation indicating viable phage. For phage titre determination, ten-fold serial dilutions of vB_SenS_ST10 were prepared in LB broth, and plaque assays were performed by plating dilutions on *S.* Typhimurium 8720/06. Plaques formed on BHI plates were counted and used to calculate the plaque-forming unit (PFU) per milliliter. Lysates from plates with high plaque counts were pooled, clarified by centrifugation, and filter-sterilized to prepare the final phage stock, which was stored at -20 °C.

Host-Range Determination

The host range of bacteriophage vB_SenS_ST10 was evaluated using a double-agar overlay plaque assay across a range of bacterial isolates listed in **Table 1**. Overnight cultures (100 µL) of each bacterial strain were mixed with soft agar, and a 10 µL aliquot of vB_SenS_ST10 was spot inoculated onto the agar plates containing bacterial lawns. Following a 24 h incubation at 37°C, plaque formation was assessed to determine the susceptibility of each isolate to vB_SenS_ST10.

Table 1. Bacterial isolates used for host-range determination, including source, isolation media, and culture conditions.

No.	Bacterial Isolate	Isolation Media	Culture Conditions
1	*Salmonella enterica* serovar Typhimurium 8720/06	BHI agar	Aerobic, 37°C
2	*S. enterica* serovar Enteritidis 81003	BHI agar	Aerobic, 37°C
3	*S. enterica* serovar Typhimurium 81205	BHI agar	Aerobic, 37°C
4	*S. enterica* serovar (SCC)	BHI agar	Aerobic, 37°C
5	*S. enterica* serovar Hadar 1477/02	BHI agar	Aerobic, 37°C
6	*S. enterica* serovar Tennessee 1328/97	BHI agar	Aerobic, 37°C
7	*S. enterica* serovar Mbandaka 739/02	BHI agar	Aerobic, 37°C
8	*S. enterica* serovar Albany 234/02	BHI agar	Aerobic, 37°C
9	*S. enterica* serovar Braenderup 9214/01	BHI agar	Aerobic, 37°C
10	*S. enterica* serovar Corvallis 8677/04	BHI agar	Aerobic, 37°C
11	*Escherichia coli* O157	MacConkey agar	Aerobic, 37°C
12	*E. coli* C1	MacConkey agar	Aerobic, 37°C
13	*E. coli* C4	MacConkey agar	Aerobic, 37°C
14	Methicillin-resistant *Staphylococcus aureus*	Mannitol Salt agar	Aerobic, 37°C
15	*S. aureus* S244	Mannitol Salt agar	Aerobic, 37°C
16	*Staphylococcus epidermidis* S168	Mannitol Salt agar	Aerobic, 37°C
17	Vancomycin-resistant *Enterococcus faecium* FM3	Azide Dextrose agar	Aerobic, 37°C
18	*E. faecalis* (ATCC® 29212™)	Azide Dextrose agar	Aerobic, 37°C
19	*Proteus vulgaris* P147	Proteae Selective agar	Aerobic, 37°C
20	*P. mirabilis* P184	Proteae Selective agar	Aerobic, 37°C
21	*Listeria monocytogenes* L55	Listeria Selective agar	Aerobic, 37°C
22	*Shigella sonnei*	XLD agar	Aerobic, 37°C
23	*Campylobacter jejuni* (ATCC® 33560™)	CCDA agar	Microaerophilic, 37°C
24	*Bifidobacterium adolescentis* (ATCC® 15705™)	MRS agar with L-cysteine	Anaerobic, 37°C
25	*B. brevis* (ATCC® 15700™)	MRS agar with L-cysteine	Anaerobic, 37°C
26	*B. bifidum* (ATCC® 29251™)	MRS agar with L-cysteine	Anaerobic, 37°C
27	*B. longum* (ATCC® 15707™)	MRS agar with L-cysteine	Anaerobic, 37°C
28	*Lactococcus lactis*	MRS agar	Anaerobic, 30°C
29	*Pediococcus* sp.	MRS agar with L-cysteine	Anaerobic, 37°C
30	*Lactobacillus reuteri* (ATCC® 23272™)	MRS agar	Anaerobic, 37°C
31	*L. gallinarum* (ATCC® 33199™)	MRS agar	Anaerobic, 37°C

Note: Abbreviations used for media include BHI (Brain Heart Infusion), XLD (Xylose-Lysine Deoxycholate), CCDA (Charcoal Cefoperazone Deoxycholate), and MRS (De Man–Rogosa–Sharpe). ATCC® denotes American Type Culture Collection, and ™ indicates trademark status. All bacteria were sourced from FAMTECH in house bacterial collection.

Efficiency of Plating (EOP)

The Efficiency of Plating (EOP) was determined to quantify the infectivity of bacteriophage vB_SenS_ST10 across different bacterial strains. Using *Salmonella enterica* serovar Typhimurium 8720/06 as the reference strain, phage titres were calculated by plating serial dilutions of vB_SenS_ST10 on both the reference and target strains. Plaques were counted following a 24 h incubation at 37 °C, and EOP values were derived by comparing the average plaque counts. The EOP value was calculated using the formula:

$$\text{EOP} = \frac{\text{Titre on target strain (PFU/mL)}}{\text{Titre on reference strain (PFU/mL)}}$$

This formula, adapted from Pelyuntha, Ngasaman [17], provided a relative measure of phage infectivity on each target strain compared to the reference.

Biofilm Inhibition and Eradication Assays

The antibiofilm activity of vB_SenS_ST10 was assessed in two separate assays, based on the protocol described by Stepanović, Vuković [18]**.** In the biofilm inhibition assay, a 1:100 diluted overnight culture of *S.* Typhimurium 8720/06 was added to microtiter wells with serial dilutions of vB_SenS_ST10. After a 48 h incubation at 37°C, wells were washed with PBS, stained with 0.25% crystal violet, and de-stained with 95% ethanol. Biofilm formation was quantified by absorbance at 595 nm, using untreated wells as negative controls. For biofilm eradication, mature biofilms were pre-formed by incubating bacterial cultures in microtiter wells for 48 h. Wells were then treated with vB_SenS_ST10 dilutions, followed by a 24 h incubation. After PBS washing and crystal violet staining, absorbance at 595 nm quantified biofilm biomass, with statistical analysis applied to assess treatment effects.

Statistical Analysis for Biofilm Inhibition and Eradication Assays

Statistical analysis for the biofilm inhibition and eradication assays was conducted using GraphPad Prism (version 10.3.1, August 2024). Data were analysed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to determine significant differences between treatments, as described by Zar [19]. Results are presented with distinct lettering to indicate statistical significance ($P < 0.05$), with letters (a, b) denoting statistically different groups.

RESULTS

Host Range Determination

The host range assay revealed that vB_SenS_ST10 demonstrated lytic activity on three specific *Salmonella* serovars: *S.* Typhimurium 8720/06, *S. enterica* (SCC), and *S.* Tennessee. Plaque formation on these strains was observed as clear zones on *S.* Typhimurium 8720/06 and as turbid plaques on *S. enterica* (SCC) and *S.* Tennessee, indicating variability in lytic efficiency. No lytic activity was observed on other bacterial isolates, including non-*Salmonella* species, suggesting a narrow host range for vB_SenS_ST10 (**Table 2**).

Table 2. Host-range determination and lytic spectrum analysis of vB_SenS_ST10 phage on a variety of bacterial isolates.

No.	Bacterial Isolate	Plaque Formation
1	*Salmonella enterica* serovar Typhimurium 8720/06	+++
2	*S. enterica* serovar Enteritidis 81003	-
3	*S. enterica* serovar Typhimurium 81205	-
4	*S. enterica* (SCC)	+
5	*S. enterica* serovar Hadar 1477/02	-
6	*S. enterica* serovar Tennessee 1328/97	+
7	*S. enterica* serovar Mbandaka 739/02	-
8	*S. enterica* serovar Albany 234/02	-
9	*S. enterica* serovar Braenderup 9214/01	-
10	*S. enterica* serovar Corvallis 8677/04	-
11	*Escherichia coli* O157	-
12	*E. coli* C1	-
13	*E. coli* C4	-
14	Methicillin-resistant *Staphylococcus aureus* (MRSA)	-
15	*S. aureus* S244	-
16	*Staphylococcus epidermidis* S168	-
17	Vancomycin-resistant *Enterococcus faecium* FM3	-
18	*E. faecalis* (ATCC® 29212™)	-
19	*Proteus vulgaris* P147	-
20	*P. mirabilis* P184	-
21	*Listeria monocytogenes* L55	-
22	*Shigella sonnei*	-
23	*Campylobacter jejuni* (ATCC® 33560™)	-
24	*Bifidobacterium adolescentis* (ATCC® 15705™)	-
25	*B. brevis* (ATCC® 15700™)	-
26	*B. bifidum* (ATCC® 29251™)	-
27	*B. longum* (ATCC® 15707™)	-
28	*Lactococcus lactis*	-
29	*Pediococcus* sp.	-
30	*Lactobacillus reuteri* (ATCC® 23272™)	-
31	*L. gallinarum* (ATCC® 33199™)	-

Note: (+++) indicates complete lysis of the bacterial lawn; (++) represents 1 to 100 plaques with complete lysis; (+) indicates 1 to 100 plaques with incomplete lysis; (-) denotes no lysis of the bacterial lawn.

Efficiency of Plating (EOP)

EOP analysis indicated a relative efficiency of 1.5 x 10^{-2} for vB_SenS_ST10 on *S. enterica* SCC compared to S. Typhimurium 8720/06 (Table 3). This low EOP value suggests reduced infectivity of vB_SenS_ST10 on *S. enterica* (SCC) relative to the reference strain *S. Typhimurium* 8720/06.

Table 3. Results of the Relative Efficiency of Plating (EOP) Procedure Between *S. Typhimurium* 8720/06 and *S. enterica* (SCC), acting as reference and target strains, respectively.

Salmonella Strain	Mean Plaque Count (PFU)	Plaque-Forming Units (PFU/mL)	EOP
S. Typhimurium 8720/06	40	4 x 10^9	1
S. enterica (SCC)	61	6.1 x 10^7	1.5 x 10^{-2}

Biofilm Inhibition and Eradication Assays

In the biofilm inhibition assay on *Salmonella enterica* serovar Typhimurium 8720/06, significant biofilm reduction was observed at titres of 10^4 PFU/mL or higher ($P < 0.05$), as indicated by **Fig. 1**. Lower titres showed minimal inhibition, suggesting a threshold concentration necessary for effective biofilm inhibition. Higher concentrations demonstrated a plateau effect, indicating a potential maximum limit for biofilm inhibition efficacy. In the biofilm eradication assay (**Fig. 2**), phage vB_SenS_ST10 did not achieve a statistically significant reduction in biofilm density at the highest concentration of 10^9 PFU/mL when compared to the control. This finding suggests that even at elevated titres, phage SvB_SenS_ST10 is ineffective at eradicating mature biofilms. The results emphasize the inherent resistance of established biofilm structures and indicate that higher phage concentrations alone may not be sufficient for biofilm removal in poultry production contexts.

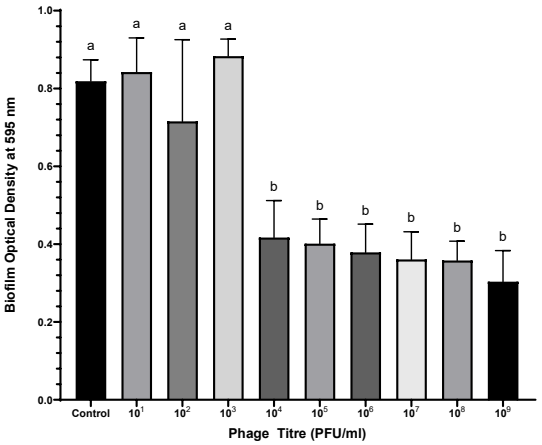

Fig. 1. Results of the biofilm inhibition assay, showing the titres necessary for significant biofilm reduction. Statistical analysis was conducted via ANOVA and post hoc (Tukey) test, with distinct letters (a, b) indicating values that are significantly ($P < 0.05$) different from each other.

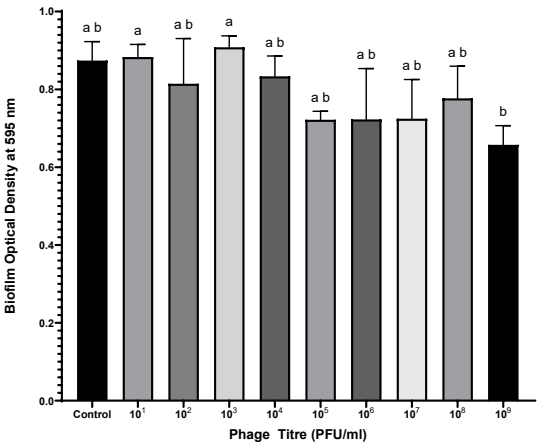

Fig. 2. Results of the biofilm eradication assay, showing titres necessary for biofilm reduction in mature biofilm structures. Statistical analysis was conducted via ANOVA and post hoc (Tukey) test, with distinct letters (a, b) indicating values that are significantly ($P < 0.05$) different from each other.

DISCUSSION

The restricted host range of vB_SenS_ST10, with lytic activity limited to *Salmonella enterica* serovars Typhimurium 8720/06, S. *enterica* (SCC), and *S.* Tennessee, highlights its potential as a targeted antimicrobial solution for *Salmonella* control in poultry production. This specificity for certain serovars may be attributed to unique receptor-binding proteins on the phage, which recognize and attach to host-specific surface structures, a critical factor in phage-host specificity [20]. Such receptor-binding specificity reduces unintended impacts on non-target bacteria, particularly beneficial gut microbiota, thus enhancing vB_SenS_ST10's suitability for biocontrol applications without destabilizing the microbial ecosystem essential for poultry health.

Phages often recognize and bind to specific components of the bacterial surface, including lipopolysaccharides (LPS) and outer membrane proteins (OMPs). LPS, which are major components of the outer membrane in Gram-negative bacteria like *Salmonella*, contain O-antigen structures that some phages specifically target for attachment [21]. Additionally, certain phages use OMPs, such as OmpC and OmpF, as receptors to identify suitable host strains. The specificity of these interactions significantly influences the phage's host range and its ability to target specific *Salmonella* serovars, further emphasizing the potential of vB_SenS_ST10 for focused antimicrobial application in poultry settings [22]. The differential plaque morphology, presenting as clear plaques on *S.* Typhimurium and turbid plaques on *S. enterica* (SCC) and *S.* Tennessee, may indicate variations in lytic efficiency across these serovars. Turbid plaques often suggest slower or incomplete lysis, which could arise from host cell resistance mechanisms or variations in receptor density and structure that affect vB_SenS_ST10's binding and replication rates [23]. Understanding these mechanisms could be instrumental in improving phage efficacy, particularly in serovars demonstrating partial resistance or reduced lytic susceptibility.

The narrow host range observed in vB_SenS_ST10 not only minimizes the risk of collateral damage to beneficial gut flora but also aligns well with current trends in phage therapy, where precision is prioritized to avoid adverse impacts on host organisms' microbiomes [24]. In the poultry industry, where maintaining a balanced microbiota is crucial for digestion, immune function, and overall health, vB_SenS_ST10's selectivity supports its candidacy as a biocontrol agent. Moreover, the lack of activity against common probiotics such as *Lactobacillus* and *Bifidobacterium* species, as observed in this study, further highlights the potential of vB_SenS_ST10 to integrate safely within established poultry farming practices without disrupting gut health or promoting dysbiosis.

Efficiency of plating (EOP) is a quantitative measure used to evaluate a phage's ability to form plaques on a target host in comparison to a reference strain [23]. A lower EOP generally indicates challenges in host compatibility or variations in receptor interactions, impacting the phage's adsorption and ability to successfully infect [25]. In the EOP analysis of phage vB_SenS_ST10, results demonstrated significantly reduced infectivity on *Salmonella enterica* (SCC) (EOP = 1.5 x 10^{-2}) relative to the reference strain, *S. Typhimurium* 8720/06, standardized to an EOP of 1. This low EOP value on *S. enterica* (SCC) suggests that vB_SenS_ST10 exhibits decreased binding efficiency or replication within this strain, potentially due to differences in surface receptors or receptor density [26]. Similar findings have been observed in *Escherichia coli* studies, where specific receptor deficiencies affect phage infectivity: *E. coli* strains lacking the *waaC* gene, responsible for partial lipopolysaccharide (LPS) production, showed an EOP as low as 2.5×10^{-4} compared to the *E. coli* K-12 strain, indicating limited receptor availability for effective phage binding [27].

Such findings emphasize the importance of EOP analysis in assessing phage adaptability across different bacterial hosts and understanding potential barriers in host recognition. For vB_SenS_ST10, targeting *S.* Typhimurium remains more efficient, which supports its use as a biocontrol agent primarily for strains closely related to *S.* Typhimurium. Further studies to explore receptor interactions and potential modifications could improve vB_SenS_ST10's lytic activity across broader *Salmonella* serovars, enhancing its utility in diverse poultry farming applications.

This study provides valuable insights into the limitations of phage vB_SenS_ST10 in managing biofilms formed by *Salmonella enterica* serovar Typhimurium. While moderate phage titres (10^4 PFU/mL) may inhibit initial biofilm formation, our findings indicate that even at the highest tested concentration (10^9 PFU/mL), vB_SenS_ST10 did not achieve significant eradication of mature biofilms, reflecting the complexity of treating established biofilm structures. The observed plateau in inhibition efficacy at higher concentrations suggests a limitation in phage access or action within the biofilm matrix, potentially due to the emergence of phage-resistant bacterial subpopulations [15, 28]. These resistant cells may protect surrounding susceptible cells, thereby enhancing the biofilm's resilience. This finding aligns with existing knowledge on biofilm heterogeneity, where diverse bacterial subpopulations contribute to the robustness and persistence of biofilms [29, 30].

This study highlights the significant challenges associated with eradicating mature biofilms, even at elevated phage concentrations. This requirement is likely due to the extracellular polymeric substance (EPS) layer that surrounds mature biofilms, acting as a barrier to phage penetration [31]. Additionally, dormant bacterial cells within these structures exhibit reduced metabolic activity, rendering them less susceptible to phage-mediated lysis [32, 33]. These protective adaptations significantly limit the effectiveness of phages in eradicating mature biofilms. Our study highlight the challenges of using phages alone for biofilm treatment, particularly for mature biofilms. The findings suggest that a combination of strategies—such as the use of phage cocktails to target various bacterial strains or incorporating EPS-degrading enzymes—may enhance the efficacy of phage-based therapies. This combined approach could potentially address the limitations observed and improve biofilm eradication outcomes.

CONCLUSION

This study evaluated the potential of bacteriophage vB_SenS_ST10 as a targeted biocontrol agent against *Salmonella enterica* serovar Typhimurium, with a specific focus on addressing biofilm formation, a persistent challenge in poultry production. Our findings demonstrate that vB_SenS_ST10 selectively targets *S. enterica* serovar Typhimurium 8720/06, *S. enterica* SCC, and *S.* Tennessee, indicating its suitability as a targeted approach for managing specific *Salmonella* strains. This specificity supports vB_SenS_ST10 as a promising alternative to traditional antibiotics, offering potential solutions amidst growing antimicrobial resistance concerns. However, the narrow host range of vB_SenS_ST10 and its limited effectiveness in eradicating mature biofilms, even at high titres (10^9 PFU/mL), highlight challenges for broader applications. To enhance its utility, future research should explore methods to expand vB_SenS_ST10's host range, possibly through phage engineering or the development of phage cocktails that target a wider array of *Salmonella* serovars. Additionally, combining vB_SenS_ST10 with biofilm-disrupting agents could improve its efficacy in eradicating mature biofilms. Such strategies could enhance the practical application of phage therapy in poultry production, contributing to more sustainable health management practices and improved food safety.

LIST OF ABBREVIATIONS

AMR – Antimicrobial Resistance
CFU – Colony Forming Units
EOP – Efficiency of Plating
EPS – Extracellular Polymeric Substance

LB – Luria-Bertani (Broth/Agar)
PFU – Plaque Forming Units
MDR – Multi-Drug Resistance
ANOVA – Analysis of Variance
BHI – Brain Heart Infusion
PBS – Phosphate Buffered Saline
XLD – Xylose-Lysine Deoxycholate (Agar)
MRSA – Methicillin-Resistant *Staphylococcus aureus*
ATCC – American Type Culture Collection
UPM – Universiti Putra Malaysia

ACKNOWLEDGMENT

The authors acknowledge Universiti Putra Malaysia for providing financial support through the Research Grant Scheme (GP-IPM/2022/9725100), which was essential for the successful completion of this research.

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