Original Article

Production and characterization of polyclonal antibody against major capsid protein of *Salmonella* bacteriophage SE-W109

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Abstract

Salmonella spp., are important foodborne pathogens that are predominantly found in poultry, swine, eggs and dairy products. Rapid detection of *Salmonella* contamination in animals and food is urgently needed to reduce the risk of infection. Bacteriophages or phages are viruses that specifically infect and kill bacteria. Our research team at the Department of Immunology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand isolated *Salmonella* phage SE-W109 that demonstrated a broad host range of infection to various serovars of *Salmonella* spp., but that did not cross-react with other Gram-negative bacteria. We then set forth to develop this phage for the rapid detection of *Salmonella* spp. contamination in food samples. However, to achieve this objective, a polyclonal antibody against *Salmonella* phage SE-W109 is required. In the present study, an optimization condition for rabbit immunization to generate an anti-phage antibody was successfully developed. Western blot analysis revealed that the generated antibody recognized the 45 kDa antigen of *Salmonella* phage SE-W109 and that it had no cross-reaction with other *Salmonella* phages or with *Salmonella* spp. Mass spectrometry analysis and PCR sequencing revealed the 45 kDa antigen to be a major capsid protein of *Salmonella* phage SE-W109. In conclusion, we report the protocol for generating a polyclonal antibody against a *Salmonella* phage and that the main antigen recognized by antiserum is a major capsid protein. The identified antibody will be used for further development of a phage-based method for detection of *Salmonella* contamination in food samples.

Keywords: Anti-phage antibody, Bacteriophage, Major capsid protein, *Salmonella* spp.

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Introduction

Salmonella spp., which is a Gram-negative bacterium, is a leading cause of food poisoning worldwide that is heavily associated with the consumption of chicken and pork meat that is contaminated with the bacteria. There are over 2,500 serovars of *Salmonella* that have been identified, and more than 50% of those serovars belong to the *S. enterica* subspecies *enterica* (Eng *et al*., 2014). Food poisoning in infants, elderly people and immunocompromised patients can produce severe symptoms (Coburn *et al*., 2007). Previous studies in Bangkok and other parts of Central Thailand have revealed a prevalence of *Salmonella* contamination in pork and chicken of 82% (33/40 samples) and 62% (25/40 samples), respectively (Niyomdecha *et al*., 2016). Therefore, an urgent need to develop a rapid and specific method for detecting *Salmonella* contamination in food samples in order to reduce the risk of infection.

Conventional culture methods have traditionally been considered the gold standard for the isolation and identification of foodborne pathogens (Zee, 1994). However, these methods are labor-intensive and time consuming because they require at least three days to produce a result. As a result, alternative rapid methods have been developed and reported (Malorny *et al*., 2004; Valadez *et al*., 2009). However, these methods require sophisticated equipment that is expensive, that requires specially trained technicians, and that is not widely available. Recently, new approaches using bacteriophages for the rapid and sensitive detection of bacteria have been developed (Hagens and Loessner, 2007).

Bacteriophages or phages are viruses that specifically infect and kill bacteria, and they are found in almost all environments on Earth (Salmond *et al*., 2015). Examples of phage-based detection methods include the development of anti-γ phage polyclonal antibodies for the rapid detection of *Bacillus anthracis* (Cox *et al*., 2015), and the generation of anti-phage A511 polyclonal antibodies for the rapid detection of *Listeria monocytogenes* (Stambach *et al*., 2015). These techniques require antibodies against bacteriophages for assay development. Although there are reports on the development of phage-based detection assays, the protocol regarding how to generate those anti-phage antibodies have not been described in detail.

Our research team previously isolated *Salmonella* phage SE-W109, which belongs to the *Siphoviridae* family and we revealed *Salmonella* phage SE-W109 to be a virulent phage which has only a lytic cycle and does not integrate its genome on the bacterial chromosome (Phothaworn *et al*., unpublished data). Some phages have both lytic and lysogenic cycles (temperate phage). Lysogenic lifestyle is a major disadvantage, as virulence-associated genes can easily be spread among bacterial pathogens by phage genome integration (Nilsson, 2014). In addition, *Salmonella* phage SE-W109 can infect and lyse all of 121 isolates of *Salmonella* spp., including serovars Enteritidis, Typhimurium, Virchow, Hadar, and Choleraesuis (Phothaworn *et al*., unpublished data), which suggests that this phage has a broad host range of *Salmonella* infection that is suitable for the development of phage-based rapid detection of *Salmonella* contamination in food. Therefore, the objective of this study was to generate and characterize a polyclonal antibody against *Salmonella* phage SE-W109 for use in the further development of a rapid and sensitive assay to detect *Salmonella* spp. contamination.

Materials and Methods

Ethical approval: Rabbit immunization to facilitate polyclonal antibody production was approved by the Kasetsart University Institutional Animal Care and Use Committee (License number: UI-01434-2558).

Bacterial strains and phages: A list of the bacterial strains and phages is shown in Table 1. Bacteriophage SE-W109 (phage SE-W109), which is specific to *Salmonella* spp., was previously isolated by our research group (Phothaworn *et al*., unpublished data). All bacterial strains were cultured at 37ºC in Trypticase soy agar (TSA) (Titan Biotech Ltd, Delhi, India). Phage were maintained in SM buffer (50 mM Tris-HCL pH 7.5, 100 mM NaCl, 8 mM $MgSO_4$, and 0.01% gelatin). Unless otherwise indicated, all reagents were purchased from Sigma-Aldrich Corporation, St. Louis, MO, USA.

Preparation of high-titer phages for immunization: High-titer *Salmonella* phage SE-W109 for rabbit immunization was propagated according to a previously described protocol (Kropinski *et al*., 2009). Briefly, 10 confluent *Salmonella* phage SE-W109 lysis plates (diameter: 150 mm) were covered with 15 ml of SM buffer for 5 h at room temperature (RT). Crude phage lysates from each plate were pooled and centrifuged at 6,000xg at 4ºC for 45 min. Supernatants were filtered through a 0.45 µm membrane filter (Whatman; Sigma-Aldrich Corporation) and then centrifuged again at 18,000xg at 4ºC for 30 min. The supernatants were gently removed and 2 ml of SM buffer was added to cover the pellet before leaving at 4ºC overnight. The next day, the bacteriophage suspension was centrifuged at 1,800xg at 4ºC for 15 min. Finally, the numbers of bacteriophages (Plaque-forming units; PFU) in the supernatant was quantitated by double-layer plaque assay (Kropinski *et al*., 2009).

Double-layer plaque assay: Double-layer plaque assay was performed as previously described (Kropinski *et al*., 2009). Briefly, 100 µl of phage suspension was mixed with 100 µl of mid-log phase of *Salmonella* host strain before incubation at 37ºC for 20 min to facilitate phage adsorption on bacterial cells. After incubation, phage-adsorbed bacteria were mixed with 3-5 ml of 0.35% melted TSA (Titan Biotech Ltd). The mixture was uniformly poured on to the surface of a 1% TSA plate. The dried plate was incubated overnight at 37ºC and the number of plaques (PFUs), which represents the number of phages, was counted the next day.

***Rabbit immunization and antibody detection*:** *Salmonella* phage SE-W109 (1×10^{12} PFU/injection) in Complete Freund's Adjuvant (Sigma-Aldrich Corporation) was used to subcutaneously inject one adult female white rabbit (age: 2 months, weight: ~2 kg) at 2 to 4 different body sites. Primary immunization was performed with Complete Freund's Adjuvant followed by subsequent boosts with Incomplete Freund's Adjuvant (Bio Basic, Inc., Markham, Ontario, Canada) for a total of 3 boosts (days 7, 15 and 30). The rabbit immunization step was a service performed by the Serology and Diagnostic Laboratory Service, Faculty of Agriculture (Kamphaeng Saen Campus), Kasetsart University, Nakhon Pathom, Thailand.

To evaluate the immune response against the injected phage antigen, 0.5-1.0 ml blood samples were collected from the rabbit prior to (day 0) and after immunization to determine the titer of anti-phage SE-W109 antibody by enzyme-linked immunosorbent assay (ELISA) according to a previously described protocol (Zaczek *et al*., 2016). Briefly, *Salmonella* phage SE-W109 was coated on an ELISA plate (Corning Inc., Corning, NY, USA) and the coated plate was blocked with 2% skimmed milk in PBS (Sigma-Aldrich Corporation) at 37°C for 1 h. Serially diluted rabbit serum (100 µl) was added to the well with subsequent incubation at 37°C for 1 h. After washing with 1x PBS (Sigma-Aldrich Corporation) containing 0.05% Tween (Sigma-Aldrich Corporation), the binding of antibodies to coated *Salmonella* phage SE-W109 antigen was detected by secondary antibody (Goat anti-rabbit antibody, 1:30,000 in 1x PBS) conjugated with alkaline phosphatase enzyme (Thermo Fisher Scientific, Waltham, MA, USA) and the enzyme substrate. The optical density (OD) of the sample was then observed at a wavelength of 405 nm.

Purification of anti-phage SE-W109 polyclonal antibody: Rabbit serum fraction that contained anti-phage SE-W109 polyclonal antibody was purified by ammonium sulfate precipitation (Wingfield, 2001) and rProtein A Sepharose™ Fast Flow Kit (GE Healthcare, Chicago, IL, USA) according to the manufacturer's instructions. The eluted anti-phage SE-W109 polyclonal antibody was dialyzed against 0.1x PBS (Sigma-Aldrich Corporation), and the final antibody concentration was determined by Bradford protein assay (Bradford, 1976).

SDS-PAGE and Western blot analysis: To identify phage proteins recognized by the antiserum, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis were performed, as previously described (Laemmli, 1970; Towbin *et al*., 1979). Phages or suspension bacterial cell pellets were suspended in a reduced sample buffer (Laemmli, 1970) before heating at 95°C for 15 min. The prepared samples were then separated by 12% SDS-PAGE for 2 h. After electrophoresis, proteins were transferred from polyacrylamide gel onto a nitrocellulose membrane using a Trans-Bot® SD Semi-Dry Transfer Cell (Bio-Rad Laboratories, Hercules, CA, USA).

Blotted proteins were detected by blocking the membrane with 5% skimmed milk (HiMedia Laboratories Pvt. Ltd., India) suspended in 1x Tris-Buffered Saline (TBS; Vivantis Technologies, Selangor, Malaysia) with 0.1% Tween 20 (TBST; Sigma-Aldrich Corporation) for 2 h, followed by incubation with anti-phage SE-W109 antiserum (1:30,000) diluted in 5% skimmed milk for 2 h. After washing with 0.1% TBST, the membrane was incubated with secondary antibodies (Goat anti-rabbit antibody, 1:1,000 in 5% skimmed milk) conjugated with horseradish peroxidase (Thermo Fisher Scientific, Waltham, MA, USA) for 1 h. After washing with TBST, the protein band of interest was detected by adding SuperSignal® West Pico Chemiluminescent Substrate (Thermo Fisher Scientific) according to the manufacturer's instructions. The chemiluminescent signal was determined using a gel imaging system positioned under charge-coupled device (CCD) camera (Syngene, Cambridge, United Kingdom).

Mass spectrophotometry (MS) analysis for protein identification: The protein band recognized by anti-phage SE-W109 antiserum (approximately 45 kDa) was identified by mass spectroscopy performed by Proteomics International, Perth, Australia. Briefly, the protein band was excised and prepared for tryptic digestion before analysis by LC-MS/MS using an Agilent 1260 Infinity HPLC System coupled with a Chipcube Nanospray Interface (Agilent Technologies, Santa Clara, CA, USA) on an Agilent 6540 Mass Spectrometer (Agilent Technologies). Digested peptides were loaded onto a ProtID-Chip-150 C18 Column (Agilent technologies) and eluted with mobile phase gradient of water, acetonitrile (Proteomics Int'l), and 0.1% (v/v) formic acid (Proteomics Int'l). The mass spectra of each peptide fragment were analyzed to identify proteins of interest using MASCOT® sequence matching software (Matrix Science Ltd, London, United Kingdom) with MSPnr100 database, Virus taxonomy.

Sequencing of amplified Salmonella phage SE-W109 major capsid DNA: The gene-encoding major capsid protein of *Salmonella* phage FSL SP-101 (GenBank accession number KC139511.1, nucleotide positions 5,779-6,828) was used as a template to design two major capsid primers: forward (5'-ATATGAGTGAAAGTGAGCGATTAGC-3') and reverse (5'-CCTGTCTTTGACATTATGTAGTCTCC-3'), corresponding to nucleotide positions 5,693-5,718 and 6,902-6,876, respectively. The designed PCR primers are located outside the coding region. Therefore, based on the positions of the PCR primer, the amplified DNA fragment is approximately 1,210-bp in length. Genomic DNA of *Salmonella* phage SE-W109 was used as a DNA template for amplification with the major capsid primers. The PCR cycle consisted of a hot start at 94°C for 3 mins, followed by 35 cycles of 94°C for 1 min, 60°C for 30 sec, and 72°C for 30 sec, before a final extension of 72°C for 5 min. After amplification, the expected PCR amplicon (1,210-bp) was detected by 1% agarose gel electrophoresis and

subjected to DNA sequencing by Axil Scientific Pte Ltd., Singapore.

Results

Generation of anti-phage SE-W109 polyclonal antibody: A rabbit was immunized with *Salmonella* phage SE-W109 (1x10^{10} PFU/injection). After three boosts (days 7, 15 and 30), antibody responses against *Salmonella* phage SE-W109 antigen was not generated or detected. A possible cause for this inability to induce antibodies against phage SE-W109 may be our use of too low a dose of antigen. For the fourth boost (day 60), we increased the phage SE-W109 antigen to 1x10^{12} PFU/injection. The antibody response gradually increased from an antibody titer below 2,000 (day 75 and day 90) to 1:100,000 on day 105 (Fig. 1A). This result suggests that the antigen dose, at least in part, contributes to antibody induction.

To confirm the result and generate more antiserum, a second rabbit was immunized with *Salmonella* phage SE-W109 (1x10^{12} PFU/infection) on day 0, followed by three boosts on days 7, 15 and 30. As shown in Fig. 1B, antibody response against *Salmonella* phage SE-W109 showed rapid anamnestic response, with the generation of a high titer (1:500,000) of anti-phage SE-W109 antibody on day 45 that remained stable until day 75. Serum samples (from days 45, 60 and 75) were collected for antibody purification. The concentration of purified antibody obtained was 1.06 mg/ml.

Specificity of anti-phage SE-W109 antibody: To investigate the specificity of anti-phage SE-W109 antibody, the antiserum was tested against phage SE-W109 and other phages isolated by our research group. These phages included 5 *Salmonella* (SE-W88, SE-W112, STm101, STm118, and STm374) and 3 *Burkholderia* (BT-57DW, BT-94DW, and BP-M3) phages. Western blot analysis revealed that the antiserum recognized *Salmonella* phage SE-W109 antigen molecular weight approximately 45 kDa. No positive band was detected on Western blot analysis of protein antigens from 5 other tested *Salmonella* phages (Table 1), which suggests no cross-reactivity to our anti-phage SE-W109 antibody against tested *Salmonella* phages. In addition, the generated antiserum showed no cross-reactivity when tested against *Burkholderia* phages (Table 1).

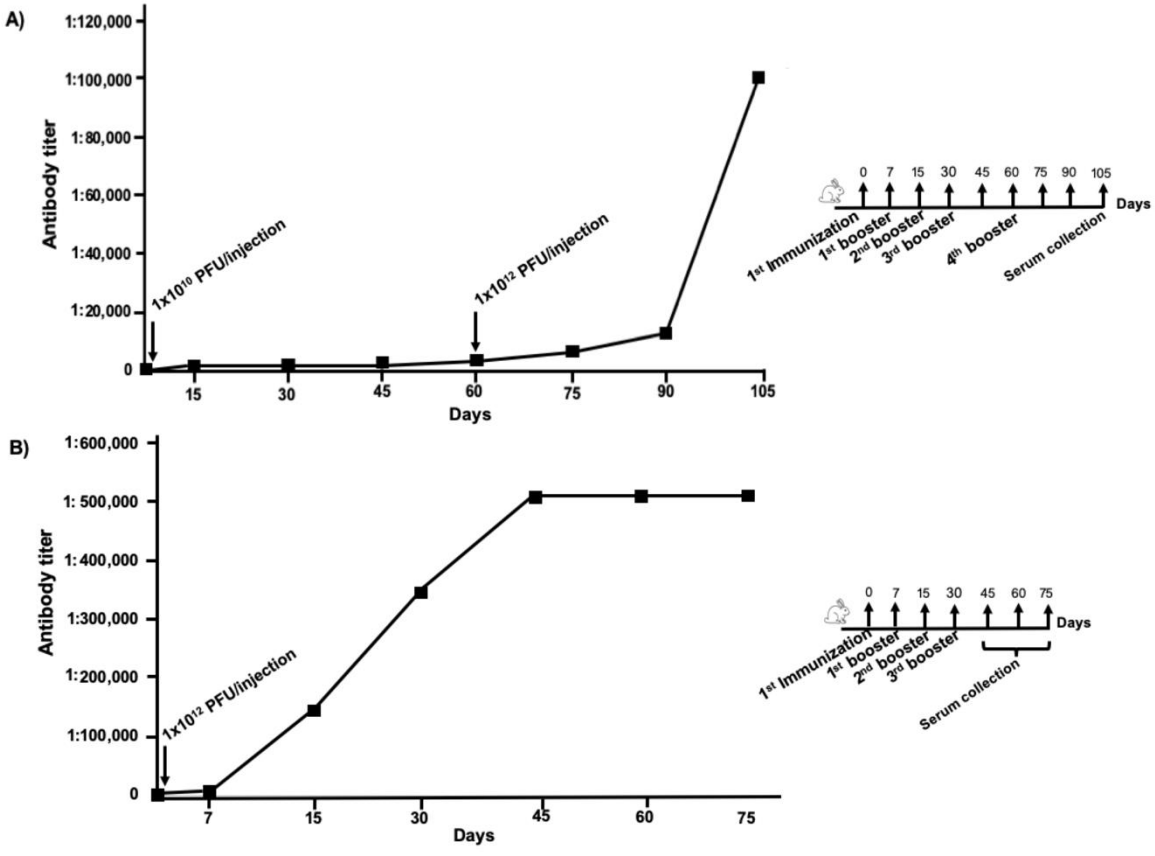


Figure 1 Antibody responses of rabbit immunized with *Salmonella* phage SE-W109. (A) A female white rabbit was immunized with *Salmonella* phage SE-W109 (1x10^{10} PFU/injection) followed by 3 boosts (days 7, 15 and 30). This regimen did not induce antibody response. The fourth boost with the amount of *Salmonella* phage SE-W109 increased to 1x10^{12} PFU/injection was performed on day 60. Serum samples containing high titer of antibody (1:100,000) (day 105) were generated. (B) Another rabbit was primarily immunized with *Salmonella* phage SE-W109 (1x10^{12} PFU/injection) followed by three boosts (days 7, 15 and 30) of 1x10^{12} PFU/injection and that rabbit also showed antibody induction. Serum samples on days 45, 60 and 75 (titer 1:500,000) were collected for antibody purification.

Table 1 Bacterial cells and bacteriophages used in this study, and Western blot analysis of antigens derived from the microorganisms immunostained with anti-phage SE-W109 polyclonal antibody.

Microorganisms	No. of isolates	Source/ reference	Western blot analysis results
Bacteria			
Salmonella enterica serovars:			
Enteritidis	5	Clinical isolate	-
Choleraesuis	5	Clinical isolate	-
Typhimurium	5	Clinical isolate	-
Virchow	5	Clinical isolate	-
Hadar	5	Clinical isolate	-
Campylobacter jejuni	5	Farm isolate	-
Campylobacter coli	5	Farm isolate	-
Listeria monocytogenes	5	Clinical isolate	-
Escherichia coli	5	Clinical isolate	-
Shigella spp.	5	Clinical isolate	-
Phages			
***Salmonella* phages**			
Phage SE-W109	1	Unpublished data	+
Phage SE-W88	1	Unpublished data	-
Phage SE-W112	1	Unpublished data	-
Phage STm101	1	Unpublished data	-
Phage STm118	1	Unpublished data	-
Phage STm374	1	Unpublished data	-
***Burkholderia* phages**			
Phage BT-57DW	1	Unpublished data	-
Phage BT-94DW	1	Unpublished data	-
Phage BP-M3	1	Unpublished data	-
Total	**59**		

In addition to testing anti-phage SE-W109 antibody specificity against phages, we further investigated the activity of the antibody against *Salmonella* spp. and other bacteria. The reason for this additional testing against bacterial pathogens is because this antibody will be used to develop an assay to detect amplified *Salmonella* phages in food samples. If it cross-reacts with *Salmonella* spp. and/or other food-borne pathogens that may be found in a food sample, it will generate a false-positive result. Table 1 shows that no positive reaction was detected for any of the tested bacteria. Taken together, the aforementioned findings and the results of Western blot analysis suggested that the anti-phage SE-W109 antibody is specific to *Salmonella* phage SE-W109.

The 45 kDa antigen is a major capsid protein of Salmonella phage SE-W109: To identify the 45 kDa protein antigen recognized by the anti-phage SE-W109 antibody (Fig. 2), the protein band was excised and subjected to mass spectrometry analysis. As showed in Fig. 3, MASCOT® data showed that the 45 kDa protein from *Salmonella* phage SE-109 matches 6 peptide fragments of a major capsid protein derived from *Salmonella* phage FSL SP-101 in the NCBI virus database (score 166), which suggests that the 45 kDa protein of *Salmonella* phage SE-109 is a major capsid protein.

To confirm that the 45 kDa antigen is a phage major capsid protein of *Salmonella* phage SE-W109, PCR analysis was undertaken using PCR primers generated from gene encoding major capsid protein sequences of *Salmonella* phage FSL SP-101. As shown in Fig. 4, the expected 1,210-bp DNA fragment was detected from DNA amplification. DNA sequencing analysis

revealed that the amplified 1,210-bp DNA showed 100% amino acid sequences similar to the major capsid protein of *Salmonella* phage FSL SP-101, which indicates that the 45 kDa antigen of *Salmonella* phage SE-W109 is a major capsid protein (Fig. 5).

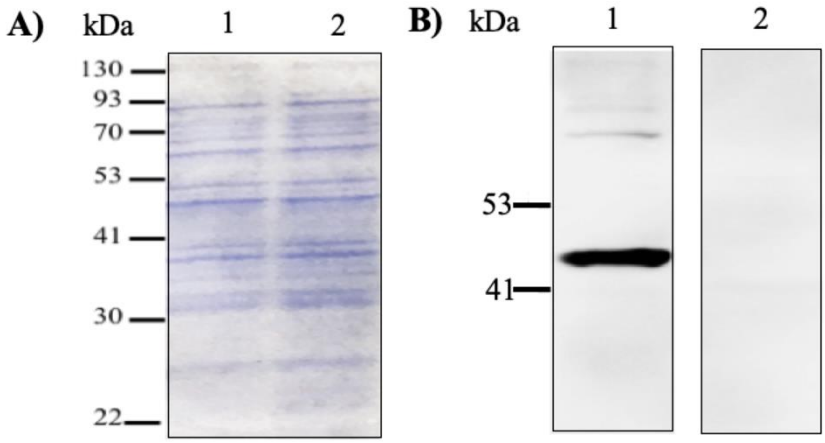
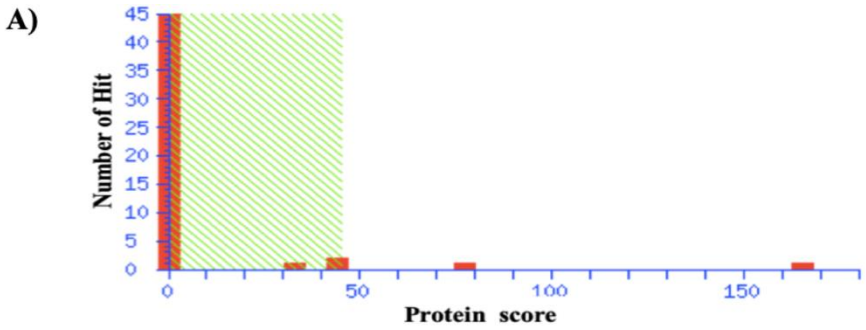


Figure 2 Western blot analysis of *Salmonella* phage SE-W109 immunostained with anti-phage SE-W109 antibody. (A) *Salmonella* phage SE-W109 lysates (Lanes 1 and 2) were separated by 12% SDS-PAGE. (B) Lane 1 shows blotted proteins from (A) immunostained with anti-phage SE-W109 antibody and detected by Goat anti-rabbit antibody conjugated with enzyme alkaline phosphatase (secondary antibody) and enzyme substrate. Lane 2 shows blotted proteins from (A) incubated with only secondary antibody to serve as a negative control.



B) **MASCOT Search Results**

Mass: 45906 Calculated pI: 4.66

Score: 166

Major capsid protein [*Salmonella* phage FSL SP-101]

Matched peptides shown in *bold:*

1 MAITTIGDIV TGNIPVLASY MTEDPVEK**TA FFDSGILTPT PYAAEIARGP**

51 **SNIANLPFWK AIDTSIEPNY SNDVYQDIAT PRAIQTGEMM AR**VAYLNEGF

101 GQADFTVELT SQNPLQSVAS RLDNFWQRQA QRRLIATALG LYNDNVSATD

151 AYHEQNDMVV DVSATLGFDA GAFIDATQTM GDALMGNGGE VLGAIAMHSF

201 VYAQARKAQL IDFIRDADNN TMFATYQGYR VIVDDSMTVV GQGAQRKFIS

251 IIFGQGAIGY GEGNPVMPLE YEREASRANG GGVETLWTRK TWLLHPFGYS

301 FTSAVITGNG TETIARSASW QDLANATNWN RVVDR**KHVPI AFLVTGVGA**

Figure 3 Identification of 45 kDa protein of *Salmonella* phage SE-109 using LC-MS/MS analysis. (A) The MASCOT® score histogram shows hits (score 166) as red bars outside the green portion, which indicates that they are considered a significant match for the major capsid protein of *Salmonella* phage FSL SP-101. (B) The matched protein fragments are highlighted in bold with sequence coverage and expected mass values.

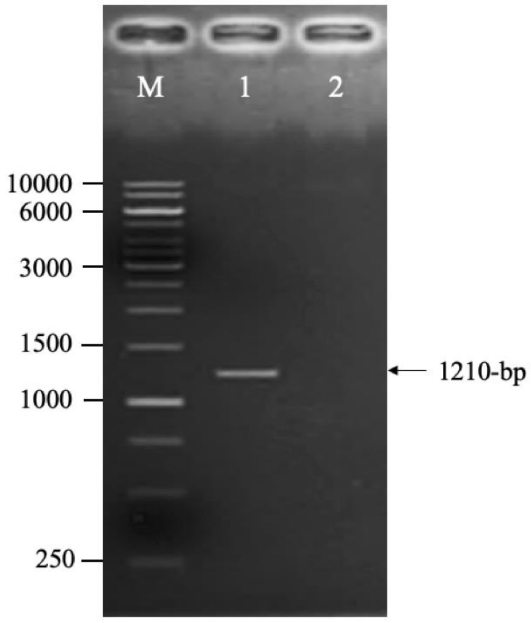


Figure 4 A 1% agarose gel electrophoresis shows *Salmonella* phage SE-W109 major capsid protein DNA. *Salmonella* phage SE-W109 DNA was amplified with PCR primers targeting major capsid protein DNA. Lane 1 shows expected 1,210-bp DNA fragment, with lane 2 used as a negative control (PCR reaction without template DNA). Lane M is a GeneRuler 1Kb DNA ladder.

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Salmonella phage FSL SP-101   1   MAITTIGNIVTGNIPVLASYMTEDPVEKTAFFDSGILTPTPYAAEIARGPSNIANLPFWK  60
                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Salmonella phage SE-W109      1   MAITTIGNIVTGNIPVLASYMTEDPVEKTAFFDSGILTPTPYAAEIARGPSNIANLPFWK  60

Salmonella phage FSL SP-101  61   AIDTSIEPNYSNDVYQDIATPRAIQTGEMMARVAYLNEGFGQADLTVELTSQNPLQSVAS  120
                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Salmonella phage SE-W109      61   AIDTSIEPNYSNDVYQDIATPRAIQTGEMMARVAYLNEGFGQADLTVELTSQNPLQSVAS  120

Salmonella phage FSL SP-101  121   RLDNFWQRQAQRRLIATALGLYNDNVAATDAYHEQNDMVVDVSATSGFDAGAFIDATQTM  180
                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Salmonella phage SE-W109     121   RLDNFWQRQAQRRLIATALGLYNDNVAATDAYHEQNDMVVDVSATSGFDAGAFIDATQTM  180

Salmonella phage FSL SP-101  181   GDALMGNGGEVLGAIAMHSFVYAQARKAQLIDFIRDAENNTMFATYQGYRVIVEDSMTVV  240
                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Salmonella phage SE-W109     181   GDALMGNGGEVLGAIAMHSFVYAQARKAQLIDFIRDAENNTMFATYQGYRVIVEDSMTVV  240

Salmonella phage FSL SP-101  241   GQGAQRKFISIIFGRGAIGYGEGSPETPLAYEREESRGNGGGVETLWTRKTWLLHPFGYS  300
                                  ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Salmonella phage SE-W109     241   GQGAQRKFISIIFGRGAIGYGEGSPETPLAYEREESRGNGGGVETLWTRKTWLLHPFGYS  300

Salmonella phage FSL SP-101  301   FTSAVITGNGIETIARSASWQDLANATNWNRVVDRKHVPIAFLVTGVGA 349
                                  |||||||||||||||||||||||||||||||||||||||||||||||||||
Salmonella phage SE-W109     301   FTSAVITGNGIETIARSASWQDLANATNWNRVVDRKHVPIAFLVTGVGA 349
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Figure 5 Amino acid sequences of *Salmonella* phage SE-W109 major capsid proteins aligned with that from *Salmonella* phage FSL SP-101 (GenBank accession number KC139511.1). The amino acid sequences identified from LC-MS/MS analysis are shown in red.

Discussion

Anti-phage SE-W109 antibody against *Salmonella* phage SE-W109 was successfully generated. In this study, we demonstrated that successful immunization required a phage concentration approximately 10^{12} PFU/infection. Concentrations of *Salmonella* phage SE-W109 lower than 10^{12} PFU/infection were unable to induce an immune response in our study rabbit. The immunization dose used in the present study was higher than the dose previously reported in *Escherichia coli* M13 phage that required $5x10^{10}$ PFU/infection (Twair *et al*., 2013), which suggests that different phages may have different levels of immunogenicity. The generated anti-phage SE-W109 antibody is specific to *Salmonella* phage SE-W109 since it shows no cross-reactivity with other phages previously isolated from our group that infect *Salmonella*, such as phages SE-W88, SE-W112, and STm101.

Although monoclonal antibody is currently in wider use than polyclonal antibody in immunodiagnostic assays, polyclonal antibody is associated with several

advantages that cannot be realized when using a monoclonal antibody. In general, polyclonal antibody has high affinity and the ability to recognize multiple epitopes, which results in more robust detection. Monoclonal antibody is specific to only one epitope, and it is more expensive than polyclonal antibody **(**Koczula and Gallotta, 2016**)**. Previous study reported the use of polyclonal antibodies to establish phage-based detection systems, such as anti-γ phage polyclonal antibodies that were developed for the rapid detection of *Bacillus anthracis* by lateral flow immunoassay combined with phage amplification technique (Cox *et al*., 2015). Further development of the anti-phage SE-W109 antibody to detect *Salmonella* contamination in chicken meat is currently ongoing in our laboratory.

Using mass spectrophotometry, we also found that the 45 kDa antigen recognized by the anti-phage SE-W109 antibody is the major capsid protein of *Salmonella* phage SE-W109. This result is consistent with the result of a previous study that found the major capsid protein of *Escherichia coli* filamentous phage fd to be a main epitope that is recognized by the antibody (Kneissel *et al*., 1999). This data suggests that the major capsid protein of the phage might be the main immunogen that induces antibody production. The major capsid protein is the main component of the phage icosahedral capsid, which functions to encapsidate the phage genome. The *Salmonella* phage SE-W109 major capsid protein consists of 349 amino acids (approximately 45 kDa). In addition, amino acid sequence alignment of *Salmonella* phage SE-W109 showed 100% similarity to that of the major capsid protein of *Salmonella* phage FSL SP-101. The *Salmonella* phage FSL SP-101 was previously isolated from a dairy farm located in New York, USA (Switt *et al*., 2013). There is no report from Thailand on the isolation of this phage. The high amino acid identity between the major capsid protein of *Salmonella* phages FSL SP-101 and SE-W109 suggested that the developed anti-phage SE-W109 antibody could recognize both of these phages. However, in term of assay development, binding of anti-phage SE-W109 antibody against phage FSL SP-101 should have no problem because the assay can be designed to remove other phages in food sample before testing.

Conclusion

We report herein a successful protocol for the generation of antibodies against *Salmonella* phage SE-W109, which could be applied for generation of antibodies against other *Salmonella* phages. The major antigen recognized by the anti-phage SE-W109 antibody was the major capsid protein of *Salmonella* phage SE-W109. The development of anti-phage SE-W109 antibody to detect *Salmonella* contamination in food samples is currently ongoing in our laboratory.

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