Bacteriophage application for biocontrolling *Shigella flexneri* in contaminated foods

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Abstract Shigellosis (bacillary dysentery) is an acute enteric infection caused by members of *Shigella* genus. It causes annual deaths of approximately five million children in developing countries. Among *Shigella* spp., *S. flexneri* causes more serious forms of dysentery than other *Shigella* species. Due to the appearance of multidrug-resistant strains of *Shigella* spp., it is necessary to find alternative antimicrobial agents. The aims of this study were the isolation of a novel species-specific phage against *S. flexneri* and to evaluate its potential and efficacy for biocontrolling of *S. flexneri* in foods. *Shigella flexneri* PTCC 1234 was used as the host strain for bacteriophage isolation from waste water. A lytic phage of the *Siphoviridae* family was isolated and designated as vB_SflS-ISF001. The phage activity remained at high levels after 1 h of incubation at − 20 to 50 °C and was fairly stable for 1 h at pH values ranging from 7 to 9. The latent period and burst size were approximately 20 min and 53 ± 4 phages per host cell, respectively. Raw and cooked chicken breast were inoculated with a predetermined amount of *S. flexneri* and subjected to biocontrol test. The results showed that using vB_SflS-ISF001 phage led to more than two logs reduction in the count of viable *S. flexneri*. It was demonstrated that using vB_SflS-ISF001 phage is of high potential for developing an alternative strategy against *S. flexneri* contamination in foodstuffs.

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Introduction

Shigella flexneri is a Gram-negative, non-motile and rod-shaped bacterium which belongs to *Shigella* genus of *Enterobacteriaceae*. *Shigella flexneri* and other members of *Shigella* genus (*S. dysenteriae*, *S. boydii* and *S. sonnei*) are causative agents of shigellosis (bacillary dysentery) (Zaidi and Estrada-García 2014). Shigellosis is responsible for annual deaths of approximately five million children in developing countries (Jafari et al. 2009). *Shigella flexneri* is endemic in many third world countries and causes more serious forms of dysentery than other *Shigella* species (Tajbakhsh et al. 2012). *Shigella flexneri* is more commonly known as responsible for shigellosis outbreaks in developing countries and also causes approximately two million infections per year worldwide (Jun et al. 2016a).

Shigella spp. are reported by the world health organization (WHO) as the third most frequently isolated bacteria from foodstuffs (WHO 2005). For example, porridge (Zhang et al. 2013), sugar snaps (Löfdahl et al. 2009) and fresh sugar pea (Muller et al. 2009) were found to be contaminated with *Shigella* spp. and reported as the suspected sources of the bacteria in Guangxi, Sweden and Denmark shigellosis outbreaks, respectively. Moreover, in 2014, chicken, beef and dairy products were reported as the sources for *Shigella* spread (Ahmed and Shimamoto 2014).

In the last few years, antibiotic resistance in bacteria has increased (Jamal et al. 2015). Although the first single antibiotic resistant *Shigella* was isolated in Japan in 1940, nowadays there are many reports on the emergence of multi-drug resistant (MDR) strains (Gu et al. 2015). Due to high similarity of *Shigella* and other members of *Enterobacteriaceae* especially *Escherichia coli*, *Shigella* strains (medical and food isolates) have gradually become resistant to most of the common recommended antimicrobial

agents (Prabhurajeshwar et al. 2015; Taneja et al. 2012). Thus, there is an urgent need to find, develop and evaluate alternative strategies to fight against *Shigella* spp., especially in food sources. Using bacteriophages is suggested as a potential strategy (Shen et al. 2012) and they have been used to control foodborne pathogens such as *Listeria monocytogenes*, *E. coli* O157: H7, *Salmonella enteritidis*, *Salmonella typhimurium*, *Campylobacter* among others (Kazi and Annapure 2015).

This study aimed to isolate, identify and characterize a novel *S. flexneri* species-specific lytic phage, and to evaluate its potential for biocontrolling of *S. flexneri* in foodstuff.

Materials and methods

Bacterial strains and growth condition

Shigella flexneri serotype 1a strain (Persian Type Culture Collection 1234) (PTCC 1234), *S. flexneri* serotype 2b (PTCC 1865), *Shigella sonnei* (ATCC 9290), *Shigella sonnei* (PTCC 1777), *Shigella dysenteriae* (PTCC 1188), *Shigella boydii* (ATCC 9207), *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883), *Streptococcus pneumonia* (ATCC 49619), *Pseudomonas aeruginosa* (ATCC 27853), *Enterococcus faecalis* (ATCC 29212), *Salmonella typhimurium* (ATCC 14028), *Proteus mirabilis* (ATCC 43071), *Staphylococcus epidermidis* (ATCC 12228), *Staphylococcus aureus* (ATCC 35933), *Streptococcus pyogenes* (ATCC 19615) were purchased from Iranian Research Organization for Science and Technology (IROST), Tehran, Iran. Of these, *S. flexneri* PTCC 1234 was used to isolate and propagate bacteriophage and also used in all other experiments. It was cultured on brain heart infusion (BHI) agar or in BHI broth (Merck, Germany) with constant shaking at 37 °C. Other strains were used for determination of the isolated phage host range.

Lytic phage isolation and purification

A number of sewage samples were collected from wastewater treatment plant in Isfahan, Iran. Then, these samples were used to isolate *S. flexneri* specific phage according to standard enrichment method with some modifications (Ghasemi et al. 2014). Briefly, 25 ml of the samples were mixed with 25 ml of an early-exponential culture of *S. flexneri* PTCC 1234 and incubated at 37 °C for 24 h with constant shaking at 100 rpm. Then, the cultures were centrifuged at 10,000×g for 10 min, and the supernatants were filtered through 0.22 μm pore size membrane (Sartorious, India) to remove bacterial debris.

Then, 20 μl of the filtrated media were spotted on the surface of solidified top agar layer (BHI with 0.7% agar) which contained 10^7 CFU/ml host cells. Following plaque appearance, the filtrated media of the previous step were serially diluted (10^{-1}–10^{-10}) in sterilized sodium chloride-magnesium sulphate (SM) buffer (100 mmol l^{-1} NaCl, 8 mmol l^{-1} MgSO4, 2% gelatin, 50 mmol l^{-1} Tris–HCl, pH 7.5) and then, 10 μl of each serial dilutions was mixed with 0.1 ml of early-exponential culture of the host cells and 5 ml of molten soft agar (BHI with 0.7% agar) at 50 °C. Finally, the mixture was decanted onto hard layer agar (BHI with 1.5% agar) to make double-layered agar plates. After 24 h, a single clear plaque was removed by a sterile Pasteur pipette and was transferred to a tube containing 500 μl of SM buffer, then subjected to homogenization and incubation (4 °C for 1 h). Afterward, 100 μl of this sample was added to 25 ml of early-log phase culture of *S. flexneri* PTCC 1234. After an overnight incubation at 37 °C, it was centrifuged (10,000×g at 4 °C for 10 min) and the supernatant was subjected to filtration (0.22 μm, Sartorious, India). Subsequently, the double-layer agar plate method was used to form single-plaque as described previously (Jeon et al. 2016). In order to ensure the purity of the phage this procedure was repeated three successive times. The phage was propagated according to Sambrook and Russell protocol (Sambrook and Russell 2001) and double-layer agar method was used to determine phage titers (Kropinski et al. 2009a).

Determination of host range

The host range was examined by the spot test method as described elsewhere (Jun et al. 2014). As described above, *S. flexneri* strains, other *Shigella* members strains and different gram positive and negative standard strains were used in this test.

Electron microscopy

The phage suspension (10^{11} PFU/ml) was concentrated by ultracentrifugation at 25,000×g for 120 min (Beckman Optima L-80 XP ultracentrifuge). The supernatant discarded and the pellet was gently resuspended in distilled water. Afterward, 10 μl of the concentrated phage was applied to a 400-mesh grid and then negatively stained with 2% uranyl acetate as previously described (Ghasemi et al. 2014). Electron micrographs were taken using a Philips HMG 400 (Netherlands) at an accelerating voltage of 100 kV.

Thermal/pH/saline stability

The stability tests were performed as previously described with some modifications (Ghasemi et al. 2014). Briefly, to evaluate the stability of the phage at different temperatures, phage suspensions (diluted in SM buffer) were placed in microtubes and incubated at − 20, 4, 25, 40, 50, 60, 70 and 80 °C for 1 h. The stability of the phage at various pH values (3–12) was assessed by incubation at 25 °C for 1 h. The effect of hypersaline condition on phage stability was determined by incubation at several concentrations of sodium chloride (1, 5, 10, 15, 20, 25, 30 and 35%) at 25 °C for 1 h. Further, bacteriophage titer was estimated using double-layer agar method (Kropinski et al. 2009a). All assays were repeated at least three times.

Determination of phage adsorption rate

Two hundred milliliters of early-log phase culture of *S. flexneri* PTCC 1234 (0.5 McFarland) was prepared and divided equally into two sterile bottles. To determine the phage adsorption rate, phage suspension was added to one bottle with a multiplicity of infection (MOI) of 0.1. The other one was inoculated with SM buffer (control bottle). Samples (1 ml) were taken every minute (up to 20 min) and phage-adsorbed cells were removed by centrifugation at $13,000 \times g$ for 30 s. Finally, the titer of the free phages in the supernatant was determined by the double-layer method (Kropinski et al. 2009a). This assay was performed in triplicate.

One-step growth curve

Burst size and latent period were assessed by the one-step growth analysis as previously described (Haq et al. 2012). Briefly, *S. flexneri* PTCC 1234 cells were harvested by centrifuging following their culture reached to appropriate optical density (OD) (OD600 nm = 0.1). Then, the pellet obtained was resuspended in 0.5 ml SM buffer and mixed with 200 μl of diluted phage (MOI = 0.01). Afterward, the mixture was incubated for 1 min and then centrifuged to remove unabsorbed phages. The pellet was resuspended in 50 ml of fresh broth medium and incubated at 37 °C. Samples (500 μl) were taken every 5 min and double-layer agar method was used to determine phage titers (Kropinski et al. 2009a). All the procedures were repeated in three independent replicates.

Bacterial lysis assay

Bacteriolytic activity was evaluated under standard laboratory conditions (SLC) according to the method described previously with some modifications (Haq et al. 2012). The early-log phase of *S. flexneri* PTCC 1234 culture at OD 600 nm = 0.1 aliquoted in sterile labeled bottles equally. The bottles were inoculated with different volumes of phage suspension to give MOIs of approximately 10 and 100. The bottles were incubated at 37 °C with a constant shaking of 110 rpm. The OD 600 absorbance was measured by Eppendorf BioPhotometer in 2 h intervals for 16 h. Bacteria-free and phage-free cultures were used as controls. All experiments were performed in triplicate.

Phage DNA isolation and restriction endonuclease analysis

A very high titer of the isolated phage suspension was prepared as described above. DNase I, RNase A (SinaClon, Iran) and proteinase K (Sigma, China) were used to degrade officious bacterial DNA, RNA, and proteins in the phage suspension, respectively. Then, the suspension was subjected to whole genome extraction according to the method explained previously (Ghasemi et al. 2014). Phage's nucleic acid was treated with the restriction enzymes including *EcoRI*, *EcoRV*, *HindIII* and *BamHI* (Thermo Fisher Scientific, US). The mixture of DNA and each endonuclease was prepared based on manufacturer's standard protocol and incubated at 37 °C for 1 h. The digested DNAs were detected by electrophoresis in 0.6% (w/v) agarose gel and visualized under UV light after staining with ethidium bromide (1 mg/ml). The sizes of different cleaved nucleic acids were estimated using SequentiX Gel Analyzer software.

Food sample preparation

Chicken is the main meat in meat-based food in the location where these experiments were done (Iran). As a result, the possibility of anthropogenic or non-anthropogenic contamination of this source with *Shigella* spp. is higher than other foodstuffs. Therefore, it was used as the food mixture in the current study. Raw and cooked chicken breasts were collected at local groceries and were preliminarily checked for contamination with *Shigella* spp. according to WHO recommended protocol (protocol number: 2010GFNLAB001) (Mikoleit 2010); then, were minced aseptically and placed in Petri dishes (5 grams) in a biosafety cabinet. Finally, the samples were kept at low temperature (4 °C) until further use.

Food experiments

The early-exponential culture of *S. flexneri* PTCC 1234 was used as a bacterial host in all food experiments. The phage (10^8 PFU/g) was applied to Petri dishes containing raw or cooked chicken breast after (in treatment

experiment) or before (in prevention experiment) inoculation with bacterial cells (10^4 CFU/g) at 4 °C to represent refrigerator temperatures.

The treatment experiment was designed to evaluate the reduction potential of the phage in the food matrix. The host bacterium (10^4 CFU/g) was pipetted onto the surface of three Petri dishes containing cooked chicken breast and other three Petri dishes containing raw chicken breast before incubating at 4 °C. Phage suspension (10^8 PFU/g) was added carefully to one of the Petri dishes after 1 h (B/P1), then, the same was performed after 24 h (B/P24) and 48 h (B/P48) of incubation for the rest of Petri dishes.

The prevention experiment was designed to evaluate preservation and inhibitory effects of the isolated phage, mimicking food conditions. For this, first, phage suspensions (10^8 PFU/g) were added completely onto the surface of three Petri dishes containing cooked chicken breast and also three Petri dishes containing raw chicken breast. The host bacterial cells (10^4 CFU/g) were added to one of the plates after 1 h incubation at 4 °C (P/B1). Afterward, the same was done for the two remained Petri dishes after 24 h (P/B24) and 48 h (P/B48) of incubation at 4 °C, respectively. Moreover, three controls including (1) negative control containing neither bacterium nor phage (C), (2) a positive control containing *S. flexneri* only (CN) and (3) a positive control containing the isolated phage (CP), were included in food experiment. After 2, 24, 48, 72, 96, 120 and 144 h samples were tested for viable bacterial counts and phage titers using pour plate and double-layer agar methods, respectively. For this, 0.5 g of each sample were transferred to sterile tubes and homogenized with 2 ml of $1 \times$ phosphate buffered saline (PBS) and 2.5 ml of SM buffer at room temperature. The liquid portion was transferred to two sterile tubes. One of them was centrifuged at $100 \times$g for 5 min, and 10 μl of the supernatant was used to count *S. flexneri* on xylose lysine deoxycholate agar (XLD agar) with pour plate method (Harley and Prescott 2002). The other one was centrifuged at $1100 \times$g for 10 min, then filtered (0.45 μm, Sartorious, India) and used to count phage numbers by double-layer agar method (Kropinski et al. 2009a). All food samples were prepared and counts carried out in triplicate.

Statistical analysis

Statistical analysis carried out by applying One-way ANOVA method on the obtained data using GraphPad Prism software, ver. 5.0 (Graph Pad Software, USA).

Results

Phage isolation and electron microscopy analysis

Seven phages were isolated from wastewater samples of wastewater treatment plant samples in Isfahan, Iran; which among them one phage with a clear plaque (0.5–2 mm in diameter) was selected for further studies (Fig. 1a) and was designated as vB_SflS-ISF001 (Kropinski et al. 2009b). Electron microscopy revealed that vB_SflS-ISF001 had an icosahedral head (64 ± 0.4 nm) which was connected directly to a non-contractile tail. The tail length and width were 112 ± 0.6 and 15.5 ± 3 nm, respectively (Fig. 1b, c). Based on its morphology, vB_SflS-ISF001 was categorized as a member of *Siphoviridae* family of the *Caudovirales* order (Ackermann 2007).

Bacteriophage host range

To evaluate the vB_SflS-ISF001 host range, its infectivity was tested on all four species of *Shigella* using spot test method. *Shigella flexneri* (n = 2), *S. sonnei* (n = 2), *S. boydii* (n = 1), *S. dysenteriae* (n = 1), *Escherichia coli*, *Klebsiella pneumonia*, *Streptococcus pneumonia*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Salmonella*
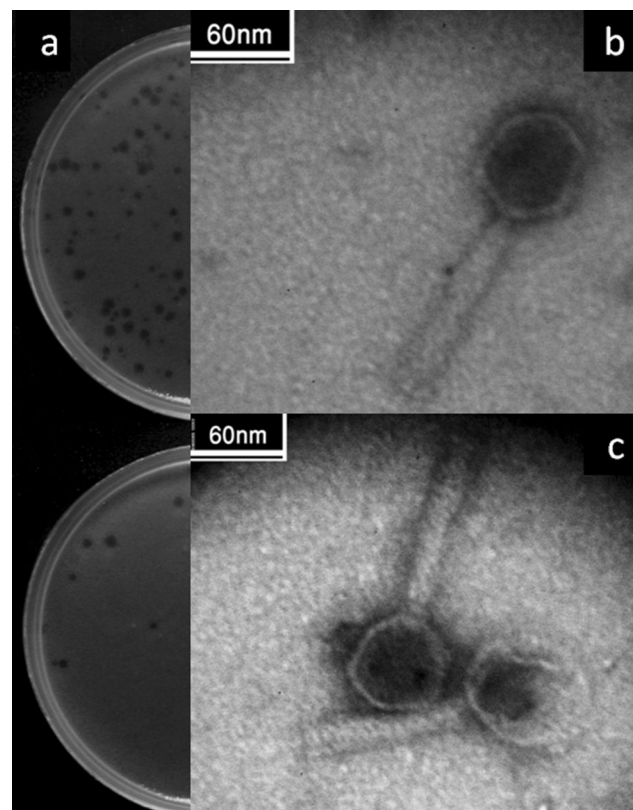

Fig. 1 Morphology of vB_SflS-ISF001 bacteriophage. **a** Phage plaques. **b**, **c** Electron micrographs of vB_SflS-ISF001

typhimurium, *Proteus mirabilis*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Streptococcus pyogenes* were used to determine the host range of phage vB_SflS-ISF001. Only *S. flexneri* PTCC 1234 and *S. flexneri* PTCC 1865 were susceptible (clear plaque formation). Also, *S. dysenteriae* PTCC 1188 was partially inhibited (turbid plaque formation).

Thermal/pH/saline stability of vB_SflS-ISF001

Phage stability was determined by monitoring the changes in vB_SflS-ISF001 titer at different pH, saline and temperature conditions. Although decreases in the titers were observed, the phage activity remained at high levels after 1 h of incubation at − 20 to 50 °C, whereas a significant decrease in the titers was observed after incubation at 60 °C ($P < 0.001$) (Fig. 2a). vB_SflS-ISF001 was fairly stable for 1 h at pH values ranging from 7 to 9, while a significant decrease in the activity was observed at pH values of 6, 11 and 12 ($P < 0.001$). No active phage was detected at pH values of 4 and 13 after 1 h incubation (Fig. 2b). As shown in Fig. 3c, while no significant reduction was observed in the phage infectivity during incubation at different saline concentrations, there was an approximately 16% increment in its activity after incubation in 1% salt solution for 1 h ($P < 0.05$).

Latent period, burst size, adsorption rate and antibacterial activity of vB_SflS-ISF001

The single-step growth experiment showed that the latent period and burst size were approximately 20 min and 53 ± 4 phages per host cell, respectively (Fig. 3a). As shown in Fig. 3b, the optical density of the bacterial culture was decreased after about 2 h, remained steady for 10 h and then increased slightly. Additionally, The adsorption rate of vB_SflS-ISF001 phage was 87.1% after 14 min at 25 °C (Fig. 3c).

Phage genome restriction pattern

Digestion patterns of vB_SflS-ISF001 genomic DNA demonstrated that it was only digested with *EcoRV* and *HindIII* (Fig. 4). According to SequentiX Gel Analyzer software data, the genome size of phage vB_SflS-ISF001 was estimated to be approximately 44,474–46,280 bp.

Efficacy of phage vB_SflS-ISF001 in biocontrolling of contaminated food

The shown results in Figs. 5 and 6 demonstrate that applying vB_SflS-ISF001 reduced the contamination rate by *S. flexneri* (in raw and cooked chicken breast) in both
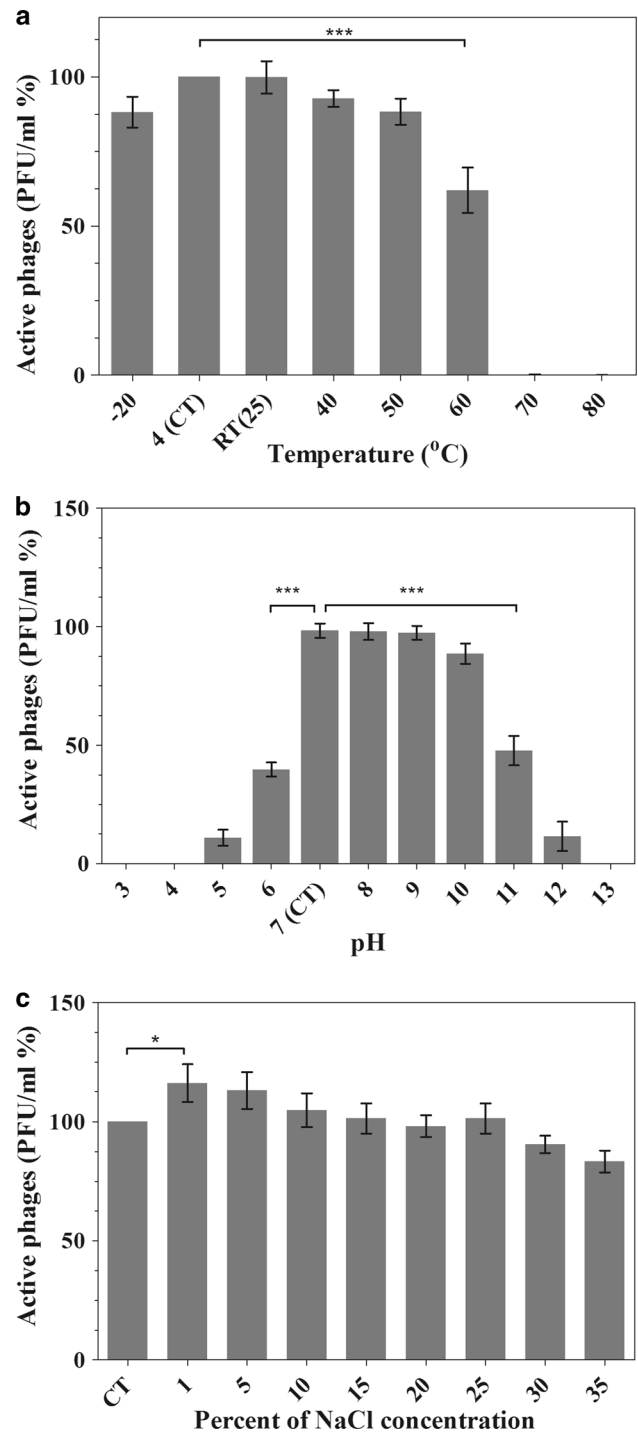
Fig. 2 The effects of different temperatures (**a**), pH values (**b**), and saline concentrations (**c**) on vB_SflS-ISF001 activity. *P* values (*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$). CT: Compare to control in each experiment. RT: Room temperature

treatment and prevention experiments. In the treatment experiment, the efficacy of phage vB_SflS-ISF001 in the reduction of the number of viable target bacterial cells (*S. flexneri* PTCC 1234) in cooked chicken breast was better than the raw chicken breast. In fact, after phage addition,
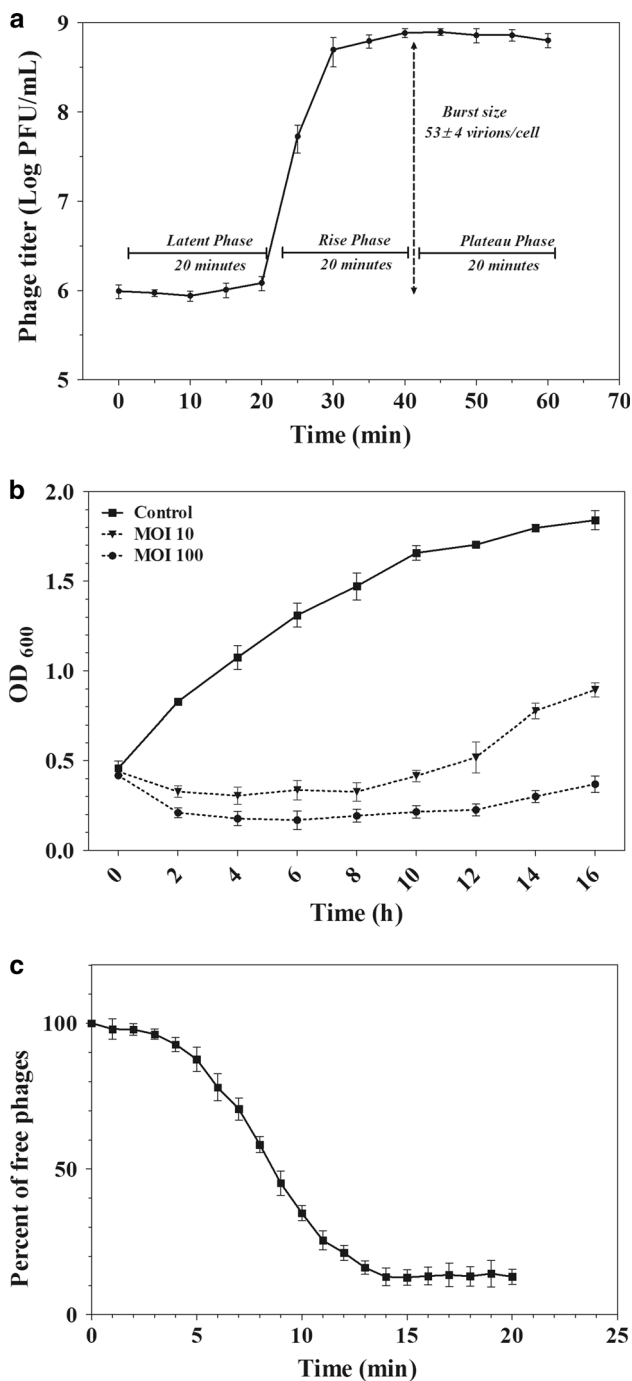
Fig. 3 One step growth curve, reduction assay test and adsorption rate of vB_SflS-ISF001. **a** Latent period and burst size. **b** Effects of mid (MOI = 10) and high (MOI = 100) titers on the growth rate of *S. flexneri*. **c** phage adsorption rate. Values are the mean ± SD (standard deviation) from triplicate assays

Fig. 4 Phage vB_SflS-ISF001 genomic DNA size. Lane M: X-large DNA marker (SinaClon, Iran); lane 1: Untreated genomic DNA; lane 2: EcoRI; lane 3: EcoRV; lane 4: HindIII; lane 5: BamHI

viable counts decreased greatly during first 24 h and then decreased slightly until 72 h (Fig. 5a, c). Phage propagation was clearly detected as the titers were increased after phage inoculation (Fig. 5b, d). The cell number of *S. flexneri* PTCC 1234 was reduced by approximately 2 logs
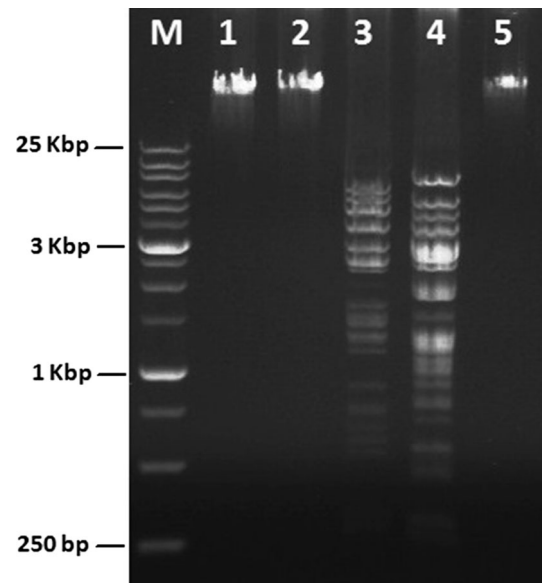
in prevention experiment within the first 24 h after host inoculation in both cooked and raw chicken breast. After 48 and 72 h, the numbers of viable *S. flexneri* cells were reduced further and were even lower than 100 CFU/g in all groups (Fig. 6a, c). Moreover, phage titer (PFU/g) was increased sharply during 24 h after addition of the bacterium (Fig. 6b, d). No significant changes were observed in the titer of *S. flexneri* PTCC 1234 cells and phage vB_SflS-ISF001 in bacterial (CB) and phage (CP) control groups, respectively.

Discussion

The *Shigella* genus is one of the most important groups of foodborne pathogens which has caused different outbreaks in many countries such as China, Sweden, and Denmark (Zhang et al. 2013). This genus is capable of contaminating various types of foods as well as drinking water and can spread quickly (Jun et al. 2014). Uncontrollable and overuse of different classes of antibiotics in agriculture, animal husbandry and clinical settings and the high potential of antibiotic resistance genes capable of spreading among different bacteria especially *Enterobacteriaceae* are the main reasons to investigate, develop and use a cost-effective alternative method (Jamal et al. 2015; Jun et al. 2014; Zhang et al. 2013).

Natural potential of bacteriophages against bacteria introduces them as possible alternative agents for antibiotics as well as a biocontrol tool in the food industry (Pérez Pulido et al. 2015). A recent study demonstrated that phage
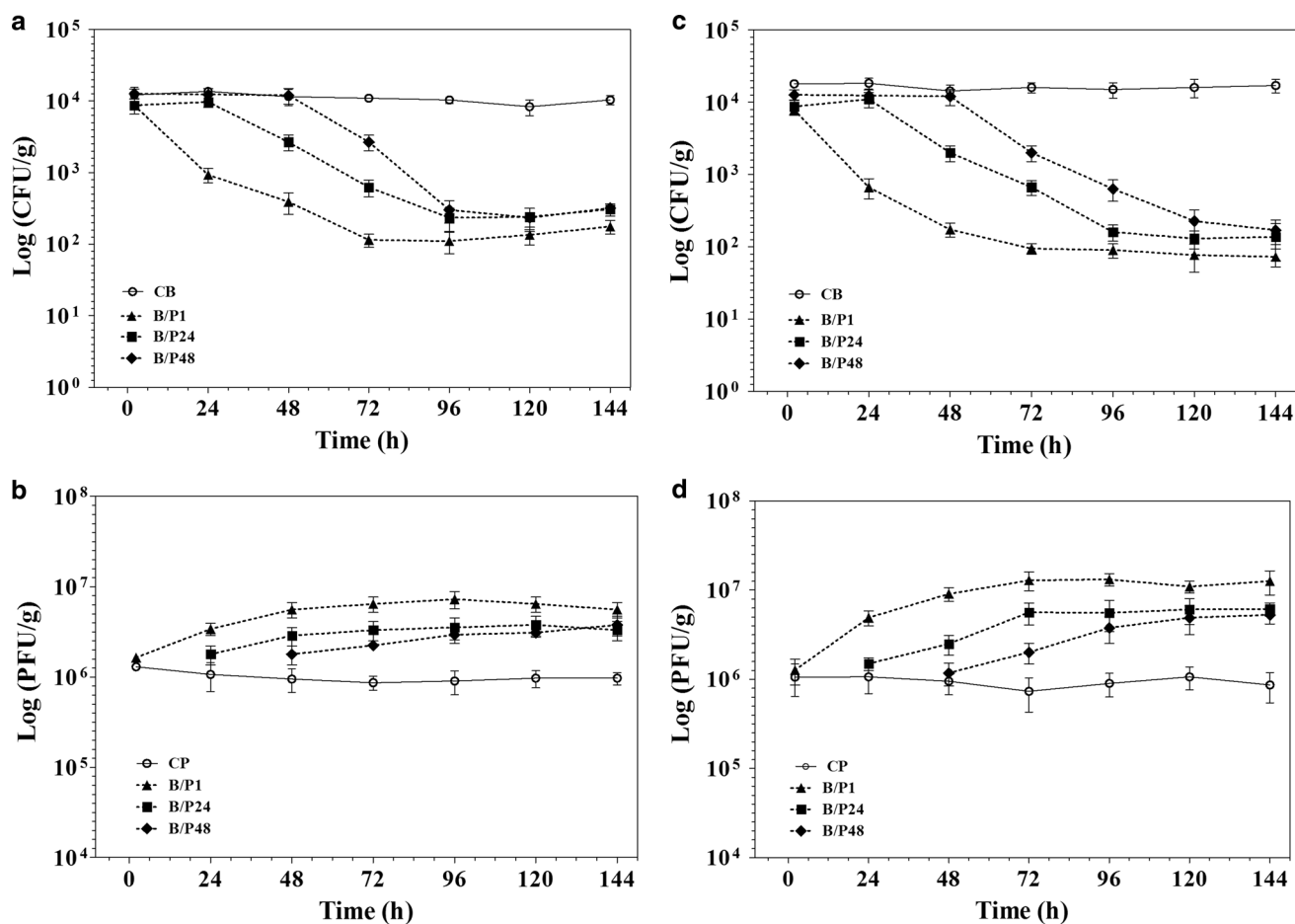
Fig. 5 Treatment effects of the phage on the viable cell numbers of *S. flexneri* in raw and cooked chicken breast (treatment experiments). **a** Viable *S. flexneri* cells in the raw chicken breast. **b** Active phages in the raw chicken breast. **c** Viable *S. flexneri* cells in cooked chicken breast. **d** Active phages in cooked chicken breast. Data represent the mean ± SD. B/P1, 24 and 48: Bacterial inoculated samples/phage was added after 1, 24 and 48 h. CB: Positive control containing *S. flexneri* only, CP: Positive control containing isolated phage vB_SflS-ISF001 only

application led to 95–99% reduction in *Campylobacter jejuni* on chicken skin (Zhang et al. 2013). Furthermore, *Salmonella* was eliminated by specific phages (Bao et al. 2015; Guenther et al. 2012; Sklar and Loerger 2001). Moreover, a phage cocktail was able to reduce *Listeria monocytogenes* contamination on different food samples (Perera et al. 2015). However, studies on biocontrol of *Shigella* by phages are rare (Zhang et al. 2013; Ahmadi et al. 2015). Therefore, the aim of this study was to isolate and characterize a novel lytic phage for *S. flexneri* and also evaluate its potential and efficacy for controlling *S. flexneri* contamination in the matrix of raw or cooked chicken breast.

Regarding the fact that *S. flexneri* is mostly a waterborne pathogen, urban wastewater samples were used for isolation of virulent phages. Phage vB_SflS-ISF001 showed a very high host specificity and was able to lyse all *S. flexneri* strains while also was able to partially inhibit *S. dysenteriae*. Although it is generally agreed that *Shigella* members and *E. coli* are sufficiently similar and recently suggested that both could be taxonomically categorized in the same species (Jun et al. 2014), vB_SflS-ISF001 did not show any lytic activity against *E. coli* strain. This phage showed a highly specific host range that makes it an appropriate agent for phage therapy and biocontrol without damaging normal flora and useful bacteria.

Shigella specific phages of *Myoviridae* and *Podoviridae* family have already been reported (Faruque et al. 2003; Jakhetia and Verma 2015; Jamal et al. 2015; Jun et al. 2014, 2016a; Zhang et al. 2013). Additionally, some phages which belong to *Siphoviridae* such as pSf-1 and pSf-2 have been well characterized earlier and are able to produce clean plaques in *S. boydii*, *S. flexneri* and *S. sonnei* cultures (Jun et al. 2013, 2016b). Although vB_SflS-ISF001, pSf-1 and pSf-2 belong to the same family but differ in morphological characteristics and genome size. The pSf-1 tail length (103 ± 6 nm) and width (13 ± 3 nm) were smaller and the head diameter
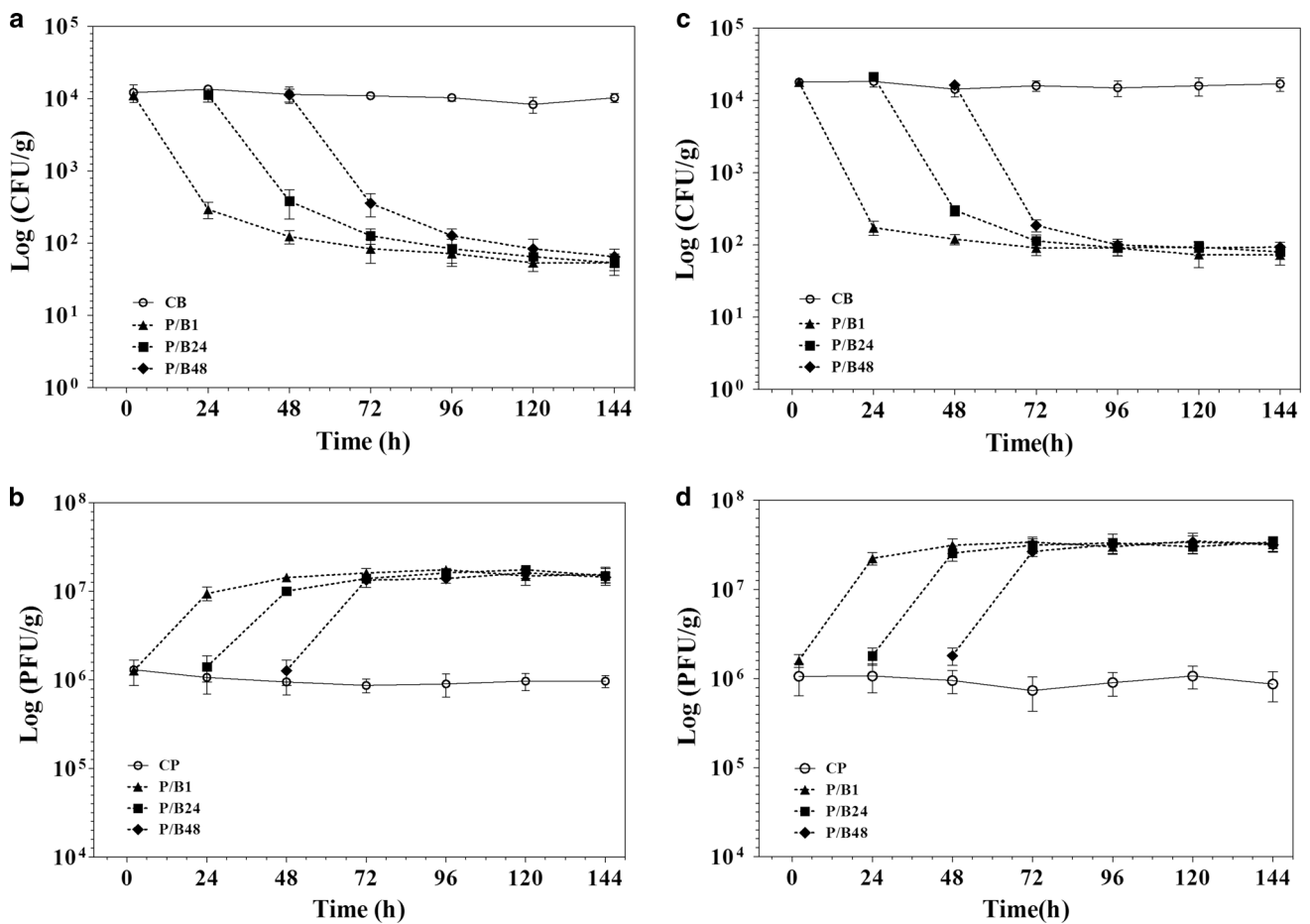
Fig. 6 Preservation-inhibition effects of the phage on the growth of *S. flexneri* in raw and cooked chicken breast (prevention experiment). **a** Viable *S. flexneri* cells in the raw chicken breast. **b** Active phages in raw chicken breast. **c** Viable *S. flexneri* cells in cooked chicken breast. **d** Active phages in cooked chicken breast. Data represent the mean ± SD. P/B1, 24 and 48: Phage inoculated samples/bacterium was added after 1, 24 and 48 h. CB: Positive control containing *S. flexneri* only, CP: Positive control containing isolated phage vB_SflS-ISF001 only

(73 ± 3 nm) was bigger than vB_SflS-ISF001 (Jun et al. 2013). In contrast, the tail length (136 ± 3 nm) and head diameter (57 ± 4 nm) of pSf-2 were respectively bigger and smaller than vB_SflS-ISF001 (Jun et al. 2016b). Moreover, pSf2 and vB_SflS-ISF001 had the same tail width (15 ± 3 nm).

The genome size of vB_SflS-ISF001 is smaller than pSf-1 (51,821 bp) (Jun et al. 2013), pSf-2 (50,109 bp) (Jun et al. 2016b) and Shfl1 (50,661 bp) (GenBank accession number: NC_015456). These results indicated that vB_SflS-ISF001 has the smallest genome size among *S. flexneri* specific *Siphoviridae* phages which had already been isolated.

Diverse phage tolerance against different pH values, temperatures, and saline concentrations has already been reported. This was examined for the phage vB_SflS-ISF001 which was stable in a significantly wide range of temperatures from − 20 to 60 °C for 1 h. It was completely inactivated after incubation at 70 °C for 1 h. Compare to

pSf-1 which is active in temperatures ranging from 4 to 50 °C, the thermal stability of vB_SflS-ISF001 was higher (Jun et al. 2013). Also, the phage was able to tolerate salt concentrations ranging from 1 to 35%. Compare to the control group, low concentrations of NaCl had a slightly positive impact on the bacteriolytic activity of the phage. The optimal activity of vB_SflS-ISF001 was recorded at pH values ranging from 5 to 12 while pSf-1 was detectable only in pH values ranging from 5 to 9 (Jun et al. 2013). In other words, vB_SflS-ISF001 showed higher activity in basic conditions compared to pSf-1. Stability results indicated that vB_SflS-ISF001 has the potential to be used in diverse environments and different foodstuffs.

One step growth curve describes different stages involved in the multiplication of phages. Large burst size could enhance the efficacy of phage therapy as well as phage-based biocontrol, as burst size is obviously related to the lytic activity and phage reproduction (Shen et al. 2012; Silva et al. 2014). Different burst sizes have been reported

for pSf-1 and pSf-2 (*S. flexneri Siphoviridae* phages) which have higher (86.86 virions/cell) and lower (16 virions/cell) burst sizes, respectively (Jun et al. 2013, 2016b). The phage vB_SflS-ISF001 has a relatively large burst size (53 ± 4 virions/cell) and short latent period (20 min); this indicates that the phage vB_SflS-ISF001 has a proper lytic activity to be used in biocontrol applications. Using *S. flexneri* PTCC 1234, the bacteriolytic activity of phage vB_SflS-ISF001 was evaluated under SLC. The results showed that the host cells growth was strongly inhibited after phage addition in different MOIs. Bacterial lysis assay also indicated that bacteriolytic activity of phage vB_SflS-ISF001 is MOI-dependent and has the highest bacteriolytic activity at MOI = 100.

This study investigated the bacterial lysis assay under SLC, yet further study is required to approve the phage potential in food biocontrol. The administrated biocontrol experiments on raw and cooked chicken breast indicate that phage vB_SflS-ISF001 has the potential to be used as a biocontrolling agent for S. *flexneri* contaminations. Phage application reduced the number of viable *S. flexneri* count at least 2 logs in both treatment and prevention experiments. This is the first study that investigated biocontrolling of *S. flexneri* using a *Siphoviridae* phage in foodstuff. As a result, no comparison was possible.

As it was shown, desirable phage stability was observed in both raw and cooked chicken breast. The newborn phage particles were released by infected cells in the first hours and increased the initial phage count for at least 1.5 logs which point toward a desirable advantage for biocontrol application. Although the phage count remained at high levels, but a significant decrease in *S. flexneri* number was recorded only up to the first 72 h. This finding could be as a result of inefficient diffusion or inhomogeneous distribution of the phage particles in food samples as well as phage trapping among food components (Guenther et al. 2012).

Conclusion

In this study, a novel *S. flexneri* specific phage (vB_SflS-ISF001) which lyses *S. flexneri* PTCC1234 and PTCC 1865 was well characterized. Its treatment and prevention potential against food contamination was evaluated. This is a fundamental research on bacteriophage application in controlling and inhibition of growth and survival of *Shigella* contamination in foodstuff. In conclusion, based on the host range, stability characterization, lysis activity, burst size and food experiments results, vB_SflS-ISF001 showed a great potential to be developed as an alternative strategy against *S. flexneri* contamination in foodstuff. Moreover, isolation of new virulent phages to make phage

cocktail is necessary and also ensures complete elimination of the pathogen in different foods.

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