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Isolation of Lytic Bacteriophages for Nanobiocontrol of Pathogenic and Antibiotic Resistant *Salmonella* Present in Poultry in Ecuador

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Abstract

The genus *Salmonella*, as many others bacteria, has been frequently reported as resistant to commonly used antibiotics in poultry, therefore, the current spread of antibiotic resistance genes in bacteria, raises doubts on the effectiveness of antibiotics in the future. One possible alternative to antibiotics is the use of bacteriophages as antimicrobial agents to control antibiotic resistant pathogenic bacteria. The main goal of this project was to isolate and use bacteriophages as a new, safe, and effective biocontrol method for treating infectious diseases caused by *Salmonella* entero-pathogena. Four lytic bacteriophages cocktails (PSEA-2, SSEA, PSIA-2, SSIA) were isolated from wastewater of poultry processing industries, to control *Salmonella enterica* subsp *entérica* serovar Enteritidis (SE), and *Salmonella entérica* subsp *enterica* serovar Infantis (SI), under laboratory conditions. We found the cocktails PSEA-2 and PSIA-2 specific for SE and SI while cocktails SSEA and SSIA also caused lysis in Pseudomona. The observations in the Transmission Electron Microscope (TEM) revealed the presence of tailed phages of the Siphoviddidae and Myoviridae families; and a polyhedral phage. We have isolated specific phages and tested *in vitro* their effectiveness at controlling *Salmonella*. Further studies are needed in order to assess the phage effectiveness *in vivo*.

Keywords: Lytic bacteriophages; Hystidine transport; Enterobacteriaceae; XLD

Introduction

Salmonellosis is a zoonotic disease causing huge economic losses worldwide, triggering a high rate of morbidity and mortality in humans; it is also one of the most common and aggressive foodborne diseases (ETAS). In Ecuador, 9908 cases of *Salmonella* were reported in 1990; by 2001 the figure suddenly increased to 18,772; this number has gradually decreased, registering in 2013 only 5972 cases [1]. However, nowadays most of the cases are not recorded because the treatments for controlling disease outbreaks are provided by private physicians (Dr. Rodrigo Paredes personal communication). Salmonellosis is caused by subspecies and serovars of the bacterium *Salmonella enterica*, which is isolated most frequently from poultry and poultry products. In recent years, according to studies of bird populations made by CONAVE (Corporacion Nacional de Avicultores Del Ecuador "Conave"), from 1990 to 2012 there has been an increase in consumption of chicken of 360%, while egg consumption has had a growth of about 60%. It is evident chicken is one of the main meat products for Ecuadorians.

The genus *Salmonella* are gram-negative bacilli of the Enterobacteriaceae family, they are none sporulating facultative anaerobes [2]. Subspecies enterica are usually found in warm-blooded animals while subspecies II, IIIa, IIIb, IV and VI are usually found in cold-blooded animals and the environment; nevertheless, all of these can infect humans [3].

An increasingly common feature of the *Salmonella* strains is that they are more resistant to antibiotics; this is due to the indiscriminate use of them in recent decades. The current spread of antibiotic resistance genes in pathogenic bacteria casts doubt on the effectiveness of antibiotics in the future. In animal production, antibiotics are not only used for therapeutic purposes, but they are also used to stimulate growth. For that reason the overuse of antibiotics should be limited and must urgently seek alternative methods to control bacterial pathogens.

One possible option against antibiotic resistant bacteria is the use of bacteriophages. Bacteriophages have been widely used to treat bacterial diseases in humans, animals and also to reduce the bacterial load in foods from animal and vegetable origin [4,5]. Phages have certain advantages over antibiotics, for example: high specificity, since only pathogenic bacteria of interest, is eliminated [6]. Bacteriophages cause no harm to endogenous individual microflora, no adverse effects on the human or animal immune system [7].

Unfortunately, antibiotics are used uncontrollably everywhere and Ecuador is not an exception. With the implementation of this project, using bacteriophages as a new, safe, and effective biocontrol method for treating infectious diseases caused by resistant pathogenic bacteria, we would introduce safer agricultural practices of food production for Ecuadorian industry.

Materials and Methods

Bacterial strains

In this study we used two epidemic strains of *Salmonella* (*Salmonella entérica* subsp *entérica* serovar Infantis (SI), and *Salmonella entérica* subsp *entérica* serovar Enteritidis (SE)), kindly donated by the Faculty of Veterinary and Animal Science at the Central University of Ecuador. Five other species of bacteria were isolated during this study and used to determine the specificity of *Salmonella* bacteriophages.

Bacteria were isolated from cloacal swab samples and stool samples collected from eight hens in a craft farm at canton Ruminahui, Pichincha province in Ecuador. Additionally, samples of residual water and different organs: gizzard, intestine, spleen and heart, were randomly taken at selected markets in Quito and Rumiñahui.

Biochemical assays

Standard bacteriological procedures were performed for bacteria management: samples were transported in 10 ml of Buffered Triptone-Water incubated for 24 h at 37°C, pre-enriched in Rappaport Vassiliadis Broth and Tetrathionate Broth Base (Difco Laboratories, United States). A loopful of each sample was streaked onto Xylose lysine deoxycholate agar (XLD) (Difco Laboratories, United States) and *Salmonella* – Shigella Agar (SS) (Difco Laboratories, United States). Following incubation at 37°C for 24 h, the suspected *Salmonella* colonies were re-streaked on XLD agar, and then incubated at 37°C for 24 h. A final identification of bacterial colonies was achieved by their biochemical reaction to: oxidase, catalase, MRVP, TSI, SIM, citrate, fermentation of glucose, maltose, sucrose and lactose. The pattern was confirmed using API 20E strips (bioMérieux, Marcy l'Étoile, France).

DNA extraction

DNA from pure bacterial culture was obtained using [8,9] modified protocols. Overnight cultures were centrifuged, pelleted and rinsed twice with TE buffer then pelleted again to re-suspend in 200 µL of TE. Bacteria were exposed to thermal shock by boiling the tubes for five minutes and immediately crushing on icy water for five minutes. After this treatment the samples were centrifuged at 12000 RPM for five minutes. Fifty four microliters of the supernatant were placed in a 200 µL microtube with 6 µL of Proteinase K (10 mg/mL). The mixture was incubated in the thermocycler C1000 Touch de Bio RadPER for 1 hour at 60°C following 15 min at 95°C. The DNA concentration was measured with a nano drop.

PCR detection

Primers with highly conserved regions to detect pathogenic *Salmonella* were selected from the literature: invA [10], fimC [11], JE0402-1 [12], and histidine operon transport [13]. The cocktail for the PCR reaction contained 4 µl of genomic DNA (100 ng/µl) obtained by heat shock treatment and 16 µl of the following solution: 5% Glycerol, 1.5 Mm MgCl2, 0.8 Mm dNTPs, 0.2 µM of each primer and 0, 05 U de Platinum Taq- polymerase (Invitrogen, United States). The reactions were performed in a C1000 Touch Thermocycler (Bio Rad) using the following thermal profile: 94°C (5 min) 35 cycles of: 94°C (30 s), 65°C (30 s) this temperature was different according to the primers used, and extension at 72°C (1 min); the program was terminated with a final 72 °C for 5 min. The PCR products were fractionated in 1.5% agarose gel at 100 V for 55 min and stained with 0.03 µl/ml of SYBR® Safe (InvitrogenTM).

Bacteriophages isolation and purification

Bacteriophages were isolated from wastewater of a medium (P) and a small (S) scale poultry processing plants (Table 1) in Pichincha-Ecuador. To increase the number of bacteriophages the samples were enriched twice with: 2.5 mL 0.5X triptose broth (HiMedia) supplemented with 10 mM de $MgSO_4$ (triptose enriched medium) and 1 mL of overnight *Salmonella* culture incubated with shaking (170 RPM) at 37°C for 24 h. The samples were then centrifuged at 4000 RPM for 30 minutes at 18°C (1248R – LaboGene Centrifuge). After the second enrichment, 5 mL of the supernatant was filtered using 0.45 and 0.20 µm syringe filters. Then 5 mL of the filtrate was mixed with 5 mL of 3 hour *Salmonella* growth (in 0.5X triptose enriched broth medium). The mixture was incubated with shaking at 37°C for 24 h. The suspension was centrifuged and filtered. This procedure was repeated six times. The resultant phage suspensions were filtered through a 0.20 µm filter and stored at 4°C. The effect of phage suspensions on *Salmonella* growth was evaluated by measuring the 3 h cultured *Salmonella* optical density (OD_o) at 600 nm and the final optical density (OD_f) of the culture after 24 h of incubation with the phage [14].

Sample	Origin	Bite
PSEA-1	Medium size poultry processing plant before bio-filter	S.E.
PSIA-1	Medium size poultry processing plant before bio-filter	S.I.
PSEA-2	Medium size poultry processing plant after bio-filter	S.E.
PSIA-2	Medium size poultry processing plant after bio-filter	S.I.
SSEA	Small poultry processing plant final steps with	S.E.
SSIA	Small poultry processing plant final steps with	S.I.

Table 1: Source of residual water from poultry industries for bacteriophages isolation and bacterial bite.

Bacteriophage titer

Bacteriophage titers were determined using the double layer agar plaque assay. Each phage suspension was serially diluted (10-1 to 10-10) in sterile distilled water. A 100 µL aliquot of each dilution together with 50 µL of cultured *Salmonella* were mixed with 2.5 mL triptose soft agar (0.4%) tempered to 45°C. The mixture was then spread uniformly through a 1.5% solid triptose agar plate and kept at room temperature for 10 min to solidify. The plaque forming unit (PFU) count was determined after overnight incubation of the plates at 37°C [14].

Host range assays

The host range of each bacteriophage´s cocktail was determined by spotting 5 µL of phage suspensions on a spread lawn of bacteria. After the spots dried, the plates were incubated at 37°C for 24 h and observed for the presence of clear lytic zones over the bacterial lawn. The bacteriophages were challenged with seven different bacteria: SE, SI, *Salmonella enterica* (unidentified strain), *E. agglomerans*, *C. freundii*, *P. fluorescens* and *E. coli*. The spot test was performed in triplicate.

Transmission electron microscopy

Negative staining electron microscopy of phages was conducted for morphological characterization. The phage suspensions were stained with 1% phosphotungstic acid (PTA) at pH 7, 2% PTA at pH 5 and 0.5% PTA at pH 4 depending on the origin of the sample. Then the preparations were observed in a transmission electron microscope at 37000 magnifications.

Results

Bacteria isolation and characterization

The genus of each control and isolated bacteria used in this study was established by their biochemic response to pre-established tests (Table 2). Bacteria of the genus *Salmonella*, *Citrobacter*, *Enterobacter*, *Pseudomona* and *Echerichia* were identified. To confirm the specie of bacteria, an AP1-20E test was performed with the same samples and controls tested previously (Table 3). The two tests: biochemical reaction and API results coincided with the following species: *Salmonella enterica*, *Citrobacter freundii*, *Enterobacter agglomerans*, *Pseudomona fluorescens* and *Escherichia coli*.

Test	S. I.	S. E.	*S. enterica*	*Citrobacter*	*Enterobacter*	*Pseudomonas*	*E. coli*
Motility	+	+	+	+	+	-	-
Catalase	+	+	+	+	+	+	+
Oxidase	-	-	-	-	-	+	-
Methyl Red	+	+	+	+	-	-	+
Voges-Proskauer	23	-	-	-	-	-	-
Indol	-	-	-	-	-	-	-
H2S	+	+	+	+	-	-	+
Citrate	+	+	-	+	+	+	-
Gas Production	-	-	-	-	-	+	-
Glucose	+	+	+	+	+	+	+
Lactose	-	-	+	+	+	-	+
Maltose	+	+	+	+	+	-	+
Sacarose	-	-	+	-	+	-	+

S.I. *Salmonella enterica* serovars Infantis

S.E. *Salmonella enterica* serovars Enteritidi

Table 2: Biochemical characterization of bacteria isolated from poultry and derivatives. Each result repeated twice. Positive reaction +, Negative reaction -.

Bacteria	Oxidase	Api 20E Code	Result	
			Bacteria	% identity
*Se. Infantis**	Negative	6704752	*S. enterica*	99%
*Se. Enteritidis***	Negative	6704752	*S. enterica*	99%
Bacteria 4	Positive	2226006	*P. fluorescens*	85%
Bacteria 1	Negative	4204112	*S.enterica*	77%
Bacteria 3	Negative	5235773	*E. agglomerans*	99%
Bacteria 2	Negative	3624512	*C. freundii*	91%
Bacteria 7	Negative	1164573	*E. coli*	81%

**Salmonella entérica* subsp *entérica* serovar Infantis

***Salmonella entérica* subsp *entérica* serovar Enteritidis

Table 3: API-20E test to confirm biochemical characterization of donated and isolated bacteria from poultry industries in Ecuador.

The pathogenicity of the *Salmonella* species was determined by PCR searching for the presence of pathogenicity genes. Those genes were

chosen from reports in the literature. The PCR products obtained from bacterial genomic DNA confirmed the presence of: invA, fimC, histidine transport operon and JEO402-1 that separated *Salmonella* from the other enterobacterias and from other none pathogenic *Salmonella* (Figure 1).

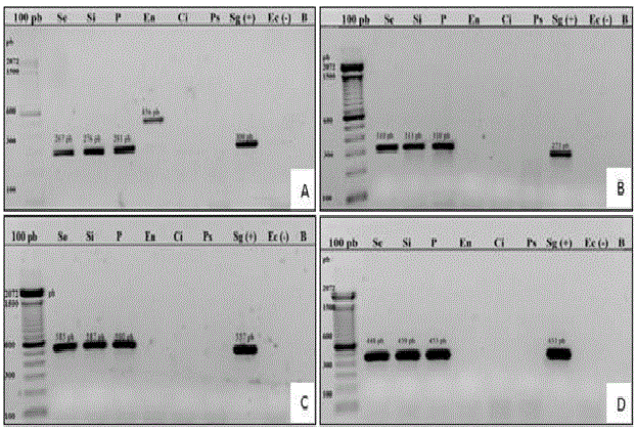

Figure 1: PCR products for *Salmonella* identification: A. primers US1f /US1r for gene invA, B. primers S212/S500 for gen fimC, C. primers GSAL-F/GSAL-R for histidine transport operón, D. primers ST11-ST15 for gene JEO402-. Where: Se: *S. enteritidis*, Si: *S. infantis*, P: *S. enterica*, En: *Enterobacter*, Ci: Citrobacter, Ps: Pseudomona, Sg (+): *S. gallinarum*, Ec: *E. coli* and B: blank.

Bacteriophages isolation

Four lytic bacteriophage cocktails (PSEA-2, SSEA, PSIA-2 and SSIA) capable of infecting SE and SI were isolated from wastewater of artisanal poultry processing plants.

All isolated cocktails reduced both SE and SI bacterial titer in liquid culture media. After overnight incubation, the OD at 600nm value was decreased in phage treated samples compared with the untreated bacterial controls (Table 4).

	After 3h	After 24h
PSEA-2	0.116	0.072
SSEA	0.103	0.069
CSE	**0.095**	**0.213**
PSIA-2	0.106	0.022
SSIA	0.109	0.075
CSI	**0.1**	**0.198**

Table 4: Effect of phage suspension on *Salmonella*. Healthy bacterial growth evaluated at three hours, decreased OD at 600 nm after 24 hrs incubation with phage suspension en relation to sterile water control (CSE abd CSI).

The phage cocktails titer was determined by double layer plaque assay: PSEA-2 ($1.4x10^8$ UFP/mL), SSEA ($1.6×10^8$ UFP/mL), PSIA-2 ($9×10^9$ UFP/mL) and SSIA ($4×10^9$ UFP/Ml). A mixture of plaques with different diameter were observed: 2, 3, 4 and 5 mm in PSEA-2; 0.5 and 1 mm plaques in SSEA; 2, 3, 4, 5, 7 and 10 mm diameter in PSIA-2 and, 1, 3 and 4 mm plaques in SSIA.

The host range of a bacteriophage is defined by the bacterial genera, species and/or strains it can lysate [15]. The lytic activity of the four bacteriophage cocktails was tested against all seven different bacteria isolated from poultry. PSEA-2 and PSIA-2 cocktails caused total lysis only in SE and SI cultures. These two phage cocktails have great potential for use in biological control of *Salmonella* due to exhibited specificity in bacterial genera, specie and sub-specie. The cocktails SSEA and SSIA besides having lytic activity against SE and SI, made turbid lytic plaques in *P. fluorescens*.

The transmission electron microscope (TEM) revealed the presence of tailed phages (Figure 2a, b, c and d) and a polyhedral phage (Figure 2e).

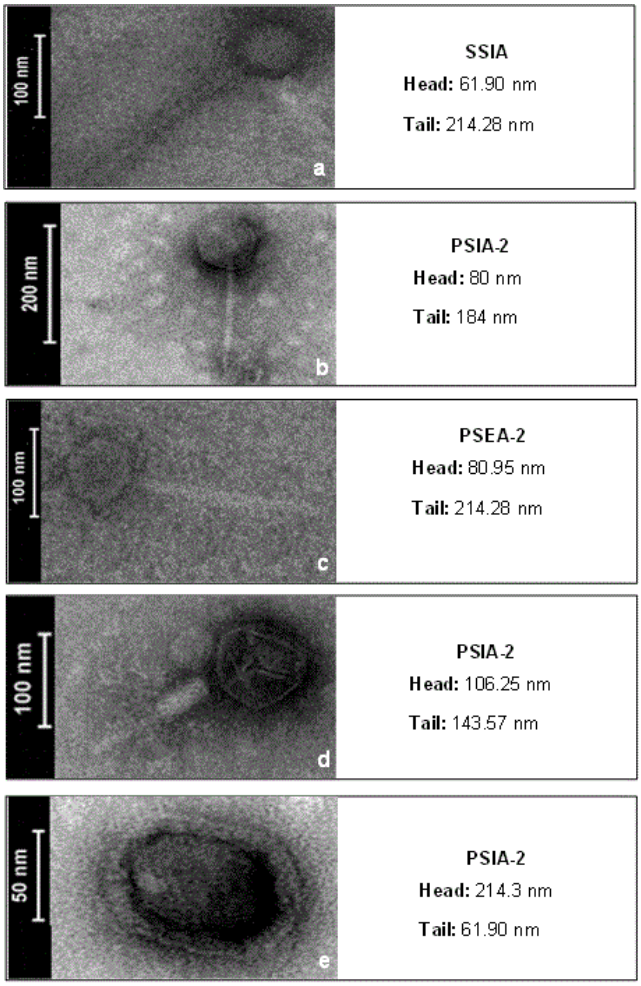

Figure 2: Bacteriophages, transmission electronmicrographies, isolated from waste water from poultry processing plants, using *Salmonella enterica* as bite.

Discussion

In this study isolated bacteria from artisanal poultry processed plants were identified, characterized and used to start the development

of a control method through the isolation and characterization of specific bacteriophages.

Five genus of bacteria were isolated from poultry and poultry related products gown and processed under artisanal conditions in Ecuador. Those genera were identified and characterized by the biochemical response of each isolate when grown under a set of culture media. The identification was confirmed analyzing the profile of each with the API-20E kit for enterobacteria identification. Both results coincided identifying *Salmonella enterica* plus four other bacterial species: *Citrobacter freundii*, *Enterobacter agglomerans*, *Pseudomona fluorescens* and *Escherichia coli*. Further characterization was done to determine pathogenicity genes in *Salmonella enterica* isolates. Specific molecular tests, confirmed the presence of genes: invA, fimbC, histidine transport and JEO-4 reported as evidence of plathogenicity in *Salmonella* species [16,17]. The genes invA and FimC are both essential in the eukaryotic cell invasion [18]. Although fimC in some publications is reported absent from *S. gallinarum* and *S. pollorum* [19], in our study fimC amplified *S. gallinarum* DNA in agreement with others [17,19] who maintain the gene is present but possibly nonfunctional.

The Histidine transport operon and JEO402-1 genes are reported as highly conserved regions in all *Salmonella* species [12,13]. The presence of these two genes in our study was distinctive of all four strains of *Salmonella* evaluated.

Our biochemical and molecular results confirm the bacteria used to isolate specific bacteriophages were indeed pathogenic *Salmonella enterica*. Therefore any phage we could isolate in this study would be applied in the control of the bacteria in further studies.

The literature recognizes as evidence of the lytic activity of bacteriophages, reduction in the optical density of host bacteria co-cultivated with lytic phages in relation to bacterial cultures without the phage [20]. In our experiments co-cultivating filtered waste water from artisanal poultry processing plants with SE and SI we saw strong evidence of bacteriophages lytic activity by the constant decrease of bacterial concentration comparing with bacterial cultures without the filtrates. The most efficient phage cocktail isolated was PSIA-2, it decreased the bacteria concentration in 381.82%, followed by PSEA - 2 with 61.11%, SSEA with 49.28% and SSIA with 45.33%.

When measuring the phage concentration of each cocktail, most of them showed a range of plaque sizes, suggesting the presence of different phage species in the samples [21].

The cocktails SSEA and SSIA besides having lytic activity against SE and SI made turbid lytic plaques in *P. fluorescens*. This is possibly because within the phage cocktail isolated from the wastewater of the small scale poultry processing plant there were bacteriophages capable of recognizing more molecules as receptors. These receptors may be present in bacteria not belonging to *Salmonella*. The more molecules a phage can recognize as receptors, the wider its host range is [22]. Also, when referring to turbid plaques or media the literature says it is because of the emergence of lysogenic bacteriophages [23]. We would need more experiments to examine those two particular cocktails, whether contains phages able to incorporate in the bacterial genome becoming lysogenic. Because of the clear bacterial specificity at the level of genera, species and subspecies, phages present in PSEA-2 and PSIA-2 have great potential for use in biological control against *Salmonella*.

Preparations from each of the cocktail of bacteriophages observed at the transmission electron microscope (TEM) revealed the presence of tailed phages and an icosahedral one. Tailed phages are the most abundant group among reported phages and represent 96% of the total; within this group 61% belong to Siphoviridae family, 25% to Myoviridae and 14.5% to Podoviridae [24]. The phages isolated in this study have a size within the range reported for Myoviridae which have icosahedral capsids with diameters larger than 80 nm and their tails are surrounded by a sheath that gives them their contractile nature. Other group of phages isolated in this study are similar to phages from the Syphoviridae having icosahedral heads with diameters ranging between 48 and 75 nm; their tails are long, flexible and non-contractile, their length varies from 110 to 300 nm [25,26] which coincide with our results. Therefore it is clear phages belonging to those families are present in our preparations. We will continue with the molecular characterization of the phages to fully identify them.

In conclusion, we have isolated four cocktails containing lytic phages specific for *Salmonella enterica* serovars Enteritidis and Infantis present in Ecuador. The cocktails have proven effective at decreasing specific bacterial growth *in vitro* and to contain bacteriophage particles as observed at the electron microscope. The specificity of the phages was confirmed by challenging them with other enterobacterias which were not affected using spot test. In such test *Salmonella enteritidis* did not survive. We have confirmed the efficiency of the phages by two *in vitro* tests. However we need further studies in order to determine the usefulness of the phages to control the bacteria *in vivo*, therefore their possible use at the farm level.

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References

1. Dirección Nacional de Vigilancia Epidemiológica (2015) Ministerio de Salud Pública. Anuario De Vigilancia Epidemiológica 1994 - 2013.
2. Caffer M, Terragno R, Binsztein N (2008) Manual de procedimientos para el diagnóstico y la caracterización de *Salmonella*.
3. Public Health Agency of Canada (2011) Public Health Agency of Canada. Retrieved from *Salmonella Enterica* Spp.
4. Goodridge L, Abedon ST (2003) Bacteriophage biocontrol and bioprocessing: Application of phage therapy to industry. Ciencia Viva 53: 254-262.
5. Hagens S, Loessner MJ (2007) Application of bacteriophages for detection and control of foodborne pathogens. Appl Microbiol Biotechnol 76: 513-519.
6. Drulis-Kawa Z, Majkowska-Skrobek G, Maciejewska B, Delattre AS, Lavigne R (2012) Learning from bacteriophages - advantages and limitations of phage and phage-encoded protein applications. Curr Protein Pept Sci 13: 699-722.
7. Marianne DP, Marion L, Colin RT, Marie-Agnès P (2014) Bacteriophages: an underestimated role in human and animal health? Front Cell Infect Microbiol 4: 39.
8. Li Q, Cheng W, Zhang D, Yu T, Ding S (2012) Rapid and Sensitive Strategy for *Salmonella* Detection Using an InvA Gene-Based Electrochemical DNA Sensor. Int J Electrochem Sci 7: 844-856.

9. Karimnasab N, Tadayon K, Khaki P, Moradi Bidhendi S, Ghaderi R, et al. (2013) An optimized affordable DNA-extraction method from *Salmonella enterica* Enteritidis for PCR experiments. Archives of Razi 68: 105-109.
10. Malorny B, Bunge C, Helmut R (1993) Evaluation of *Salmonella* spp. specific primer-sets for the validation within the Food PCR project. Federal Institute for Health Protection of Consumers and Veterinary Medicine.
11. Drahovská H, Turna J, Piknová L, Kuchta T, Szitásová I, et al. (2001) Detection of *Salmonella* by polymerase chain reaction targeted to fimC gene. Biologia 56: 611-616.
12. Aabo S, Rasmussen OF, Rossen L, Sørensen PD, Olsen JE (1993) *Salmonella* identification by the polymerase chain reaction. Mol Cell Probes 7: 171-178.
13. Cohen ND, Neibergs HL, McGruder ED, Whitford HW, Behle RW, et al. (1993) Genus-specific detection of *salmonellae* using the polymerase chain reaction (PCR). J Vet Diagn Invest 5: 368-371.
14. Rahaman MT, Rahaman M, Rahaman MB, Khan M, Hossen M, et al. (2014) Poultry *Salmonella* Specific Bacteriophage Isolation and Characterization. Bang J Vet Med 12: 107-114.
15. Kutter E (2009) Phage Host Range and Efficiency of Plating. In: Clokie M, Kropinski A (eds.) Bacteriophages: Methods and Protocols. Volume 1: Isolation, Characterization and Interactions. Humana Press, pp: 141.
16. Rahn K, De Grandis SA, Clarke RC, McEwen SA, Galán JE, et al. (1992) Amplification of an invA gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. Mol Cell Probes 6: 271-279.
17. Jones MA (2013) Fimbriae and Flagella of *Salmonella enterica*. In: Barrow PA, Methner U (eds.) *Salmonella* in Domestic Animals.
18. Galán JE, Ginocchio C, Costeas P (1992) Molecular and functional characterization of the *Salmonella* invasion gene invA: homology of InvA to members of a new protein family. J Bacteriol 174: 4338-4349.
19. Cohen HJ, Mechanda SM, Lin W (1996) PCR amplification of the fimA gene sequence of *Salmonella typhimurium*, a specific method for detection of *Salmonella* spp. Appl Environ Microbiol 62: 4303-4308.
20. Henry M, Biswas B, Vincent L, Mokashi V, Schuch R, et al. (2012) Development of a high throughput assay for indirectly measuring phage growth using the OmniLog(TM) system. Bacteriophage 2: 159-167.
21. Talledo M, Gutiérrez S, Merino F, Rojas N (1998) Detección, cuantificación y caracterización morfológica de bacteriófagos indicadores de Vibrio cholerae. Revista Peruana de Biología, 5: 90-97.
22. Kutter E, Sulakvelidze A (2004) Bacteriophages: Biology and Applications. CRC Press, USA.
23. Terzaghi BE, Sandine WE (1975) Improved medium for lactic streptococci and their bacteriophages. Appl Microbiol 29: 807-813.
24. Ackermann H (2009) Phage classification and characterization. In: Clokie M, Kropinski A (eds.) Bacteriophages: Methods and Protocols. Volume 1: Isolation, Characterization and Interactions. Clifton, USA, pp: 127-140.
25. Hatfull G (2012) The secrets lives of Mycobacteriophages. In: Hatfull ML, Szybalski W (eds.) Advances in virus research, Bacteriophages, Part A. Elsevier, USA, pp: 189.
26. Fokine A, Rossmann MG (2014) Molecular architecture of tailed double-stranded DNA phages. Bacteriophage 4: e28281.

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