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# Isolation, Molecular Characterization and Insight into the Genome Sequence of *E. coli* Bacteriophage ADB-2 from Poultry Fecal Sample

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## Abstract

Here we describe the isolation of few *E. coli* phages specifically ADB-2 and *S. typhimurium* bacteriophages from poultry fecal sample, its molecular characterization, genome sequencing and functional annotation. Escherichia phage ADB-2 was isolated from a chicken fecal sample. It is a virulent phage and shows effective inhibition of *Escherichia coli* strains. An antibiogram of the natural host of Escherichia phage ADB-2 showed that the host was sensitive against norfloxacin and gentamicin and that it demonstrated higher resistance against cotrimoxazole and oxytetracycline. The data generated from the genomic library contained 229,781 reads and 45,496,800 nucleotide bases with an average read length of 198 bases. The assembly using Newbler version 2.6 generated a 50,552 bp long single chromosome. The genome annotation and comparative analysis of the genome using Rapid Annotation using Subsystem Technology showed that the phage genome comprised of 46% GC with 76 predicted coding regions and 2 RNA genes. The genome confirmed the presence of functional genes related to phage structure and packaging machinery, phage neck protein, tail structure for host interaction, phage DNA synthesis and host lysis. The complete genomic analysis of phage ADB-2 provides new insights into its characteristics and interactions with the host bacterium *Escherichia coli*. The complete genome sequence of the *Escherichia* phage ADB-2 genome can be accessed under the Gen Bank accession number JX912252.1.

**Keywords:** Drug resistant bacteria; Biosafety; Bacteriophage; Poultry fecal; Antibiogram; Whole Genome Sequencing; Annotation

## Introduction

*Escherichia coli* and *Salmonella* have been recognized as zoonotic pathogens in animals and humans and are primary causes of reported food poisonings worldwide, with massive outbreaks occurring in recent years [1-3]. *E. coli* is found to be of economic significance in poultry causing chicken mortality [4]. Antibiotics are widely used to augment the development of animals in livestock husbandry. The use and exploitation of antibiotics in animals have led to appearance of resistant bacteria [5,6], leading to concerns about the potential impact of antibiotic. Poultry meat is one of the most popular foods, often found to be contaminated with pathogenic bacteria. These infectious bacteria enter the human food chain through contamination of the environment via poultry products and fecal matter released by the infected chickens [7]. Conventional treatment of antibiotics has been reported less effective in recent years due to the emergence of antibiotic resistance in the causative agent [8]. Improvements in safety on poultry farms are becoming expensive and difficult [9], thereby leading to need to find a cost-effective way of preventing the infection of poultry with pathogenic bacteria [10]. Use of bacteriophages is gaining attention as an alternative method for preventing the bacterial contamination [11,12]. It is considered as a potential alternative biocontrol method to inhibit the pathogen [13,14]. Virulent phages cause bacterial host cell lysis and not only function to control bacterial populations but also can be used as indicators of bacterial contamination in fecal samples [15,16] and as a potential tool for identifying specific bacterial strains [17,18]. A multivalent virulent bacteriophage would be a good selection for phage therapy because of its wide host range.

Phages have already been tested against salmonellae and other pathogens in humans and animals, with advantages compared to antibiotics [19-22]. Nevertheless, due to their high specificity, they can preferably produce in their natural hosts [23,24], as the pathogenic hosts might release cell debris, endotoxins and exotoxins which would interfere when found in the crude phage lysate [21,25-27]. The issue can be slightly overcome by use of nonpathogenic host thus eliminating the interference risk of a pathogen [23,26,28]. Consequently, it would also simplify the purification, leading to reduced cost and increased safety of phage preparations. However, this seems to be a difficult approach due to the rare presence of multivalent phages [21,23,28,29]

The aim of this study was to isolate, enrich and purify bacteriophages from poultry fecal matter and further determination its molecular weight and polyvalence. Finally, detection of specific genes and mutation in them, if any, by whole genome sequencing of the bacteriophage DNA.

## Materials and Methods

### Isolation, enrichment and purification of bacteriophages

**Host bacterial cultures:** Salmonella strains and *Escherichia Coli* strain used as phage hosts were obtained from the Microbial Type Culture Collection (Table 1). Freeze-dried primary cultures were reconstituted in Luria-Bertanibroth overnight at 37˚C. Secondary culture was prepared by inoculating on nutrient agar slants cultures, grown overnight at 37˚C, sealed and stored in dark at 5˚C. Subcultures were produced weekly or biweekly as needed.Nutrient agar plates were

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| Serial No. | Host Name | MTCC No. |
|---|---|---|
| 1. | *Salmonella typhimurium* | 98 |
| 2. | *Salmonella enteritidis* | 3219 |
| 3. | *Salmonella typhi* | 733 |
| 4. | *E.coli* | 1610 |
| 5. | *E.coli* | 739 |
| 6. | *E.coli* | 476 |
| 7. | *Entero bacteraerogens* | 2822 |
| 8. | *Protease vulgeris* | 742 |
| 9. | *Pseudomonas aerugenosa* | 741 |
| 10. | *Salmonella paratyphi A* | 735 |
| 11. | *Shigellaflexneri* | 1457 |
| 12. | *S. aureus* | 96 |

**Table 1:** List of Bacterial strain collected from MTCC.

checked for purity and uniformity and working cultures prepared by transfer of single isolated colonies to LB broth.

**Collection of poultry excreta:** 32 Samples of 30–50 g poultry excreta were collected from nine different poultry farms in Gujarat, India, in sterile sample collector (Hi-media) in December 2008; February 2009; May 2009 and June 2009. Samples were collected in various forms such as freshly laid fecal sample, dry fecal sample and semi-solid fecal samples under guidance of veterinary doctor and curator of poultry farm.

**Isolation of bacteriophage:** A suspension of fecal contents (1:9, w/v) was prepared in SM buffer and incubated at 40˚C for 24 hrs with gentle agitation followed by centrifugation at 13,000×g for 10 minutes to remove bulk debris. The supernatant was then filtered through a 0.22 µm-pore size membrane filter to remove any remaining bacterial cells. This filtered supernatant was added to equal quantity of double strength T-broth (g/L): Peptone,10g; Meat extract, 3 g; NaCl, 5 g; Glucose, 1 g; $CaCl_2$, 0.2 g; $MgSO_4$, 0.5 g; pH 7.4 (with HCl or NaOH) and incubated at 37˚C for 24 hrs with shaking. Resultant growth culture was treated with chloroform to release phage encoded in it. Then centrifuged at 13,000 × g for 5 min and supernatant was passed through 0.22 µm filter, this filtrate was used in Double Agar Layer (DAL) plaque assay against the host bacterium. For plaque assay, enrichment of phage lysate was performed by adding to equal quantity of T-broth inoculated with 1ml of overnight grown culture of bacterial host and incubated at 37˚C for 24 hrs under agitation. Chloroform was added and the suspension was centrifuged at 10,000 × g for 15 min, supernatant was collected and filtered through 0.45 µm membrane filter. This filtrate was used to prepare serial dilutions of phage lysate with sterile saline dilution. 0.5 ml of young culture of host and 0.5 ml of each dilution were transferred to 10 ml of melted top agar tubes. Test suspensions were mixed thoroughly by vortexing and dispensed uniformly over the surface of 20 ml of hard nutrient agar in 96 mm-diameter plate for each dilution. Melted agar overlays were allowed to harden at room temperature then plates were inverted and incubated overnight at 37˚C. Plaque were counted or individually sub-cultured as appropriate to the enumeration or isolation protocol.

**Enrichment of isolated bacteriophage:** Fecal samples were reconstituted in SM buffer and treated with chloroform and stored at 40˚C overnight to allow larger suspended sediments to settle out. These crudely clarified samples were then passed through 0.22 mm syringe filters. Lytic phages were selectively enriched by mixing filtered effluent with double strength trypticase soy broth inoculated with an actively growing culture of host. (*E. coli* MTCC 739, *S. typhimurium* MTCC 98 and *S. typhi* MTCC 733). After overnight incubation at 35˚C, chloroform was added and samples were stored at 50˚C. Enriched samples were tested by Double Agar Layer (DAL) plaque assay against individual respective host. For isolation of phages, enriched samples were diluted in sterile saline in 10-fold series and used in DAL plaque tests at appropriate dilutions to yield separated individual plaques suitable for single- plaque transfer. All phage titers reported in this thesis are mean values of at least 2 measurements. Phage isolates (*E. coli, S. typhimurium* and *S. typhi phage*) were tested against 13 different strains of taxonomically related bacteria. Phage lysate were allowed to enrich into T-broth inoculated with respective bacterial test hosts and incubated overnight at 37˚C. Plaque formation, indicating a susceptible host for the respective phage, was assessed after 24 hrs. Polyvalence of isolated Bacteriophage was also tested by Spot assay method.

## Isolation of *E. coli* and *S. typhi* as a natural host of phages

Fecal samples were collected from chicken in different district of Gujarat state. 9 Poultry fecal sample from which bacteriophage were isolated used for the isolation of their host bacteria. Suspension of fecal contents (1:9, w/v) was prepared in SM buffer (50 mM Tris-HCl [pH 7.5], 0.1 M NaCl, 8 mM MgSO4.7H2O, and 0.01% gelatin) and incubated at 40˚C for 24 hrs with gentle agitation to allow bacteria to elute into the buffer. This suspension was then subjected to centrifugation at 5,000 × g for 10 min to remove bulk debris. For the isolation of *E. coli* and *S. typhi* Brilliant Green Agar (BGA) and Mac Conkey's Lactose Agar (MLA) selective medium were used. Agglutinability of the suspected *Salmonella* culture with *Salmonella* polyvalent 'O' sera (SPAN Diagnostic Ltd, INDIA) was tested using agglutination test. Eosine Methylene Blue and Mac Conkey's agar were used for the preliminary identification of suspected *E. coli* isolates. Further identification was carried out on the basis of morphology, cultural characteristics, biochemical reaction. Growth curve pattern for all the 37 bacterial isolates were carried out by inoculating loop full culture in to Nutrient broth medium. Cultures were grown overnight in CMB medium and next day transferred to CM agar plates and slants. They were incubated at 37˚C for 24 h. In order to study diversity and identification of the organisms, the isolates were inoculated into different biochemical media supplemented with NaCl (5%, w/v) and incubated at 37˚C for 24-72 h and results were subsequently observed. The biochemical tests performed includes catalase and oxidase test, urea hydrolysis, indole production, $H_2S$ production, ammonia production, nitrate reduction, triple sugar iron reaction and sugar fermentation test following standard protocol [30].

## Determination of antibiogram of isolated natural host bacteria by disc diffusion technique

The sensitivity of organism against 32 antimicrobial agents was determined by the disc diffusion technique. 0.1 ml overnight grown culture of isolated bacteria (1 x $10^7$ cfu / ml) mixed with 15 ml melted N-Agar at 45-50°C and poured on base agar containing petriplate. After that antibiotic discs were applied with sterile forceps to ensure even contact with the medium. All plates were incubated at 37˚C for 24 hrs.

## 16S rRNA gene sequencing and phylogenetic classification

The PCR primers used to amplify 16S rDNA fragments were the bacteria - specific primers (Lane, 1991) F27 (5'-AGA GTT TGA TCM TGG CTC AG-3'); and R1492 (5'-ATA GGY TAC CTT GTT ACG ACT-3'). PCR amplification 25 µl reaction mixture was performed by

Thermal Cycler (ABI, USA) using the following program: Denaturing at 95˚C for 5', followed by 30 cycles of 30s of denaturing at 95˚C, 30s of annealing at 50˚C and 2' of elongation at 72˚C with a final extension at 72˚C for 10'. The PCR product (1400 bp) was cleaned by using a Qiagen DNA Gel Extraction Kits (QIAGEN, CA). Sequencing was performed for all isolates (purified PCR product) using above primer with an ABI Prism 310 Genetic analyser (Applied Biosystems Inc., CA) using Big Dye Terminator (version 3.1) at Animal Biotechnology laboratory, AAU, Anand, Gujarat, India. The 16s rDNA sequence for Isolates were initially analyzed at NCBI server (http://www.ncbi.nlm.nih.org) using BLAST tool and corresponding sequences were downloaded. Evolutionary history was inferred using the Neighbor-joining method [31]. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distance used to infer the phylogenetic tree [32].

## Whole genome sequencing of Escherichia phage adb-2 and functional annotation

Genomic DNA was extracted from the stock by the alkaline lysis method. The whole-genome sequencing of *Escherichia* phage ADB-2 was performed using Ion Torrent PGM (Ion 200-bp sequencing kit) (Life Technologies). The generated reads were De Novo assembly using Newbler version 2.6, followed by genome annotation and comparative analysis of the genome were done using Rapid Annotation using Subsystem Technology (RAST) [33].

## Results and Discussion

Out of 32 samples tested for the presence of phages of *E. coil* and *S. typhimurium* and *S. typhi* by double agar layer plaque method, 5 samples showed the presence of *E. coli* phages named as ADB-1, ADB -2, ADB -3, MH -2 and MH-5 isolated from the sample AM-2 , AM-4, AM-5 , MH-2 and MH- 4 respectively. 4 samples showed the presence of *S. typhimurium* phage named as SM-3, SM-5, LHSM- 4 and LHSM - 5 isolated from sample DK-3, DK-5, LH-4 and LH-5 respectively (Table 2).

Standard DAL method used for the determination of Phage titers, performed in triplicates showed that Dh-T1 sample had the highest pfu while ST-4 was found to be having the lowest pfu value (Table 3). The data of pfu is very useful for the further experiment and also very useful for the determination of infectivity of phage to their respective host bacteria.

13 bacterial strains were tested for sensitivity against the isolated phages by e Spot Test and DAL method. Ec-2, ADB-2, Ec-5 and ST-4 isolates of phage were found host specific while SM-3 and SM-5 were found polyvalent bacteriophage, SM-3 was having the capacity to

| Name of Poultry farm | Sample code | *E. coli* phage | *S. typhimurium* phage | *S. typhi* phage |
|---|---|---|---|---|
| Dhebakuva – 1 | DK-1 | - | - | - |
| | DK-2 | - | - | - |
| | Dk-3 | - | + | - |
| Dhebakuva –2 | DK-4 | - | - | - |
| | Dk-5 | - | + | - |
| Dharmaj | DJ-1 | - | - | ND |
| | Dj-2 | - | - | ND |
| Ankalav | Ak-1 | - | - | ND |
| | AK-2 | - | - | ND |
| | AK-3 | - | - | ND |
| Anand Agriculture University | LH-1 | - | - | - |
| | LH-4 | - | + | - |
| | LH-5 | - | + | - |
| | CH-2 | - | - | - |
| Rajkot | RJ-1 | - | - | ND |
| | RJ-2 | - | - | ND |
| | RJ-3 | - | - | ND |
| | RJ-4 | - | - | ND |
| Mustafa Poultry farm AMRELI | AM-1 | - | - | - |
| | AM-2 | + | - | - |
| | AM-3 | - | - | - |
| | AM-4 | + | - | + |
| | AM-5 | + | - | + |
| | AM-6 | - | - | - |
| Mahuva Poultry farm – 1 | MH-1 | - | - | - |
| | MH-2 | + | - | - |
| | MH-3 | - | - | - |
| | MH-4 | + | - | - |
| Mahuva Poultry farm – 2 | MH-5 | - | - | ND |
| | MH-6 | - | - | ND |
| | MH-7 | - | - | ND |
| | MH-8 | - | - | ND |

**Table 2:** Isolation of Coliphage from poultry fecal samples.

| Dilution | No. of plaque (Y) | | | N=Y/VX (pfu/ml) | Mean (pfu/ml) | Mean (pfu/ml) |
|---|---|---|---|---|---|---|
| | Plate -1 | Plate -2 | Plate -3 | | | |
| $10^{-1}$ | Uncountable | Uncountable | Uncountable | - | - | - |
| $10^{-2}$ | Uncountable | Uncountable | Uncountable | - | - | - |
| $10^{-3}$ | Uncountable | Uncountable | Uncountable | - | - | - |
| $10^{-4}$ | Uncountable | Uncountable | Uncountable | - | - | - |
| $10^{-5}$ | Uncountable | Uncountable | Uncountable | - | - | - |
| $10^{-6}$ | Uncountable | Uncountable | Uncountable | - | - | - |
| $10^{-7}$ | Uncountable | Uncountable | Uncountable | | | |
| $10^{-8}$ | Uncountable | 293 | 421 | $714 \times 10^{8}$ | $7.14 \times 10^{10}$ | $8.5 \times 10^{11}$ |
| $10^{-9}$ | 467 | 351 | 259 | $718 \times 10^{9}$ | $72 \times 10^{10}$ | |
| $10^{-10}$ | 33 | 102 | 48 | $121 \times 10^{10}$ | $121 \times 10^{10}$ | |
| $10^{-11}$ | 0 | 16 | 6 | $14 \times 10^{11}$ | $140 \times 10^{10}$ | |
| $10^{-12}$ | 0 | 0 | 0 | - | | |
| $10^{-13}$ | 0 | 0 | 0 | - | | |
| $10^{-14}$ | 0 | 0 | 0 | - | | |

**Table 3:** pfu for ADB-2.

infect *E. coli, S. typhimurium* and *S. enteritidis,* while SM-5 infected *S. typhimurium* and *S. enteritidis* (Table 4). Due to the ability to infect various species of bacteria, it has been concluded that the isolated bacteriophages SM-3 and SM-5 can become an effective agent in the effort to reduce the incidence of salmonellosis in poultry. Further study of SM-3 and SM-5 will also provide the basis for future experiments for the phages as a potential indicator and biocontrol agent in poultry [34,35].

For the treatment of various infectious diseases antibiotics have remained as the "magic bullet" since very long period of time. Available antibiotics have successfully been used for the treatment of countless number of infections in humans and animals. But eventually many antibiotics have lost their effectiveness [36]. Bacteria naturally developed various resistant mechanisms against the available antibiotics and this process still continuous to develop more and more resistance.

The isolates when tested against different antibiotics showed that the *E. coli* host for ADB-2 phage was sensitive against Tetracyline, Lomefloxacine, Sparfloxacine, Amoxyciline, Ofloxacine (Zone diameter ≥ 2.0 cm), showed intermediate resistance against Oxytetracyline, Penicillin, Oxacillin, Cephalothin, Amkalin (Zone of diameter ≤ 0.5 cm) and highly resistance against Bacitracin, Cephaloridine, Cephadroxil, Ceftazidime, Ampicilline, Lincomycin, Co-trimoxazole. *S. typhi*ARJ-2 phage was observed to be sensitive against Lomefloxacine, Sparfloxacine, Amoxyciline, Ofloxacine, Ceftriaxone (Zone diameter ≥ 2.0); it showed intermediate resistance against Bacitracin, Polymyxin, Ampicillin, Erythtromycin, Clindamycin, Penicillin, Oxacillin, (Zone of diameter ≤ 0.5 cm) and higher resistance against Cephaloridine, Cephadroxil, Ceftazidime, Lincomycin, Co-trimoxazole, Oxytetracylin (Table 5). Studies have reported that the major cause of antibiotic resistance is the poultry feed as supplements and uncontrolled antibiotic usage [37]. Determination of antibiogram and drug resistance pattern of the isolates will provide the clinician an idea regarding therapeutic schedule needed in individual cases. The data of antibiogram is also a useful tool to devise a comprehensive chemoprophylactic and chemotherapeutic drug schedule and doses of antibiotic within a geographical area.

| Host | *E. coli* phage | | | | *S. typhimurium* phage | *S. typhi* phage |
|---|---|---|---|---|---|---|
| | EC-2 | ADB-2 | EC-5 | SM-3 | SM-5 | ST-4 |
| *E. coli 1610* | +/-c | -/+t | +/-c | -/+t | - | ND |
| *E. coli 739* | +tc | +c | +tc | ND | ND | - |
| *E. coli 476* | +c | +c | +tc | - | - | ND |
| *E. coli ADB-2* | - | +tc | ND | ND | ND | ND |
| *E. aerogens* | - | - | - | - | - | - |
| *S. typhimurium* | - | - | - | +/-c | +/-c | - |
| *S. enteritidis* | - | - | - | +/-t | +/-t | ND |
| *S. typhi* | - | - | - | - | - | +tc |
| *S. peratyphi A* | - | - | - | - | - | ND |
| *Shigellaflexneri* | - | - | - | - | - | ND |
| *P. vulgaris* | - | - | - | - | - | ND |
| *P. aeruginosa* | - | - | - | ND | ND | ND |
| *S. areus* | - | - | - | - | - | ND |

Note: + Sign indicates plaque formation; +/- indicates small or diffuse plaque; -/+ indicates a very small plaque; - indicates no plaque formation; c indicates a clear plaque; t indicates a turbid plaque; tc indicates a turbid plaque with a clear center; ND indicates "no description" due to apparent loss of infectivity in storage following isolation

**Table 4:** Host range of isolated bacteriophages.

| Serial No. | Antibiotics | Concentration (micrograms) | *E. coli* (Inhibition zone diameter in cm.)* |
|---|---|---|---|
| 1 | Tetracycline | - | 2.00 |
| 2 | Bacitracin | - | R |
| 3 | Cephaloridine | - | R |
| 5 | Polymyxin | 300 | 0.9 |
| 6 | Neomycin | 30 | 1.4 |
| 7 | Novobiocin | 30 | 1.1 |
| 8 | Kanamycin | 30 | 1.3 |
| 9 | Amikacin | 30 | 1.6 |
| 10 | Lomefloxacin | 10 | 2.5 |
| 11 | Cephadroxil | 30 | R |
| 12 | Sparfloxacin | 5 | 2.3 |
| 13 | Netillin | 30 | 1.4 |
| 14 | Ceftazidime | 30 | R |
| 19 | Cefaperazone | 75 | 1.2 |
| 20 | Ampicillin | 10 | R |
| 21 | Cephotaxime | 30 | 1.4 |
| 22 | Augmentin | 30 | 1.3 |
| 23 | Cefuroxime | 30 | 1.2 |
| 24 | Ceftriaxone | 30 | 1.9 |
| 25 | Amoxycillin | 10 | 2.2 |
| 26 | Erythromycin | 15 | 0.7 |
| 27 | Clindamycin | 02 | 1.1 |
| 28 | Gentamycin | 10 | 1.4 |
| 29 | Ofloxacin | 05 | 2.4 |
| 30 | Lincomycin | 02 | R |
| 31 | Ciprofloxacin | 05 | 1.6 |
| 32 | Tobramycin | 10 | 1.3 |
| 33 | Cotrimoxazole | 25 | R |
| 34 | Norfloxacin | 10 | 1.4 |
| 35 | Oxytertracyline | 30 | 0.3 |
| 36 | Amoxyclav | 30 | 0.6 |
| 37 | Penicillin | 10 (Units) | 0.5 |
| 38 | Oxacillin | 1 | 0.3 |
| 39 | Cephalothin | 30 | 0.4 |
| 40 | Amkalin | 30 | 0.4 |

*R instead of numerical value in the diameter column stands for 'Resistant'

**Table 5:** Antibiogram Of isolated *E. coli* ADB-2 host bacteria.

Nine samples out of 32 samples from which the phages had been isolated were used for theisolation of host bacteria. Total six *E.coli* isolates and four salmonella host bacteria were isolated based on their colony characteristics and cell morphology (Table 6). Organisms grown on selective and differential medium, showed characteristic pigmentation of colonies like white, dirty white, metallic green sheen, black cantered and light yellow. The isolate ADB-2 produced green metallic sheen colony on EMB Agar and SMARJ-2 produced dark black colony on WB Agar. Size of colonies varied from small to moderate to large having smooth or rough texture with even, uneven, margins and circular, rhizoid and irregular forms. Microscopic observations revealed that all bacterial isolates were Gram negative organisms.

Biochemical characterization is the primary tool for identification of microorganisms [30]. Tables 7 and 8 show the biochemical test and sugar fermentation capacity of the suspected *E. coli* isolate. All the isolates except MH-3 and LH-6 were found to be positive for the catalase production. IMViC the key test for the identification of coli-form groups of organisms was also performed to confirm. All

the isolates gave deamination and nitrate reduction test positive except DK-5. All the isolates showed negative for Gelatin hydrolysis. Isolate ADB-1, MH-3 and DK-5 hydrolyzed starch. All the isolates ferment glucose and fructose, maltose and sucrose. Lactose and xylose fermented by ADB-2 and DK-5 only. All the isolates showed 35˚C as optimum temperature for their growth and grew at salt concentration up to 1%.

| Poultry farm | Sample | *E. coli* | Salmonella |
|---|---|---|---|
| Mustafa Poultry farm AMRELI | AM – 2 | ADB – 1 | SMARJ – 1 |
| | AM – 4 | ADB – 2 | SMARJ – 2 |
| | AM – 5 | - | - |
| Mahuva Poultry farm | MH – 2 | MH – 3 | - |
| | MH – 4 | MH – 4 | - |
| Dhebakuva – 1 | DK – 3 | - | SMDK – 3 |
| Dhebakuva – 2 | DK – 5 | DK – 5 | - |
| Anand Agricultural University | LH – 4 | LH – 6 | SMLH – 4 |
| | LH – 5 | - | - |

**Table 6:** Isolation of Coliphage from poultry fecal samples.

| Biochemical Test | | ADB-1 | ADB-2 | MH-3 | MH-4 | DK-5 | LH-6 |
|---|---|---|---|---|---|---|---|
| Catalase | | + | + | - | + | + | - |
| Indole production | | + | + | + | + | - | + |
| Methyl Red | | + | + | + | + | + | + |
| Vogues Proskauer | | - | - | - | - | + | - |
| Citrate Utilization | | - | - | + | - | - | + |
| Deamination | | + | + | + | + | - | + |
| Nitrate Reduction | | + | + | + | + | + | + |
| Urea | | + | - | - | - | + | - |
| Hydrolysis | Starch | + | - | + | - | + | - |
| | Casein | - | - | + | - | - | - |
| | Gelatin | - | - | - | - | - | - |
| Triple sugar iron | Slant | *AC* | *AC* | *AC* | *AC* | *AC* | *AK* |
| | Butt | *AC* | *AC* | *AC* | *AK* | *AC* | *AC* |
| | $H_2S$ | - | - | - | - | - | - |
| | Gas | + | + | - | - | + | + |

Note: + Positive test; - , Negative test; AC= Acidic reaction; AK = Alkaline Reaction

**Table 7:** Biochemical test of *E. coli* isolates.

Salmonella genus contains more than 2000 species. Among the 4 isolates from salmonella phage positive sample, all the isolates except SMDK-3 showed catalase and methyl red positive. SMARJ-2 and SMLH-4 also utilized citrate. All the isolates except SMARJ-2 gave deamination test positive. In Sugar fermentation test all 4 isolates ferment glucose and fructose. In case of xylose, only SMARJ-2 and SMDK-3 were found to be positively utilizing. 35˚C is the optimum temperature for all except SMARJ-2 that showed growth at 25˚C to 55˚C temperature range. Selected isolate ADB-2 and SMARJ-2 was identified on the basis of 16S rDNA gene homology. The use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been used due to several reasons like its presence in almost all bacteria, the conserved function of the 16S rRNA gene and the size of the 16S rRNA gene is large enough for analysis and interpretation

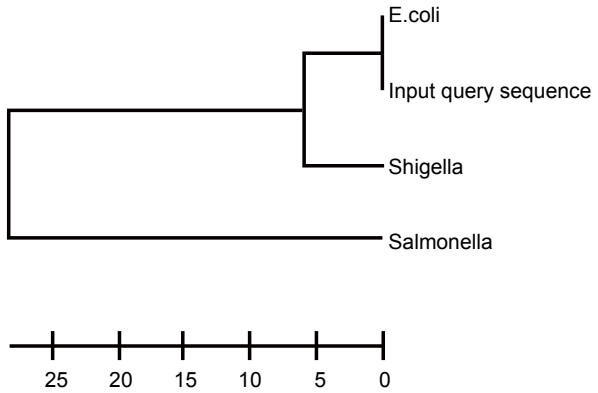


**Figure 1:** The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 62.11780002 is shown. The phylogenetic tree was linearized assuming equal evolutionary rates in all lineages The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. Codon positions included were 1st + 2nd + 3rd + Noncoding. All positions containing gaps and missing data were eliminated from the dataset. There were a total of 431 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4.

Model: Nucleotide: Maximum Composite Likelihood
[1] = *Shigella*; [2] = Input query sequence; [3] = *E. coli;* [4] = *Salmonella.*

| Biochemical Test | | ADB-1 | | ADB-2 | | MH-3 | | MH-4 | | DK-5 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Acid* | *Gas* | *Acid* | *Gas* | *Acid* | *Gas* | *Acid* | *Gas* | *Acid* | *Gas* |
| Sugar 1% (w/v) | Glucose | + | + | + | + | + | + | + | + | + | + |
| | Fructose | + | + | + | + | + | + | + | + | + | - |
| | Maltose | + | - | + | + | + | + | + | + | - | + |
| | Sucrose | + | + | + | + | + | - | + | - | + | + |
| | Lactose | + | - | + | + | - | - | - | - | + | + |
| | Xylose | - | - | + | + | - | - | + | - | + | + |
| Temp. | 25$^0$C | - | | + | | - | | + | | - | |
| | 35C | + | | + | | + | | + | | + | |
| | 55$^0$C | - | | - | | - | | - | | - | |
| Salt conc. | 0.5% | + | | + | | + | | + | | + | |
| | 1.0% | + | | + | | + | | + | | + | |
| | 2.0% | - | | - | | + | | + | | + | |
| | 5.0% | - | | - | | - | | - | | - | |

**Table 8:** Sugar fermentation and Biochemical test of *E. coli* isolates.

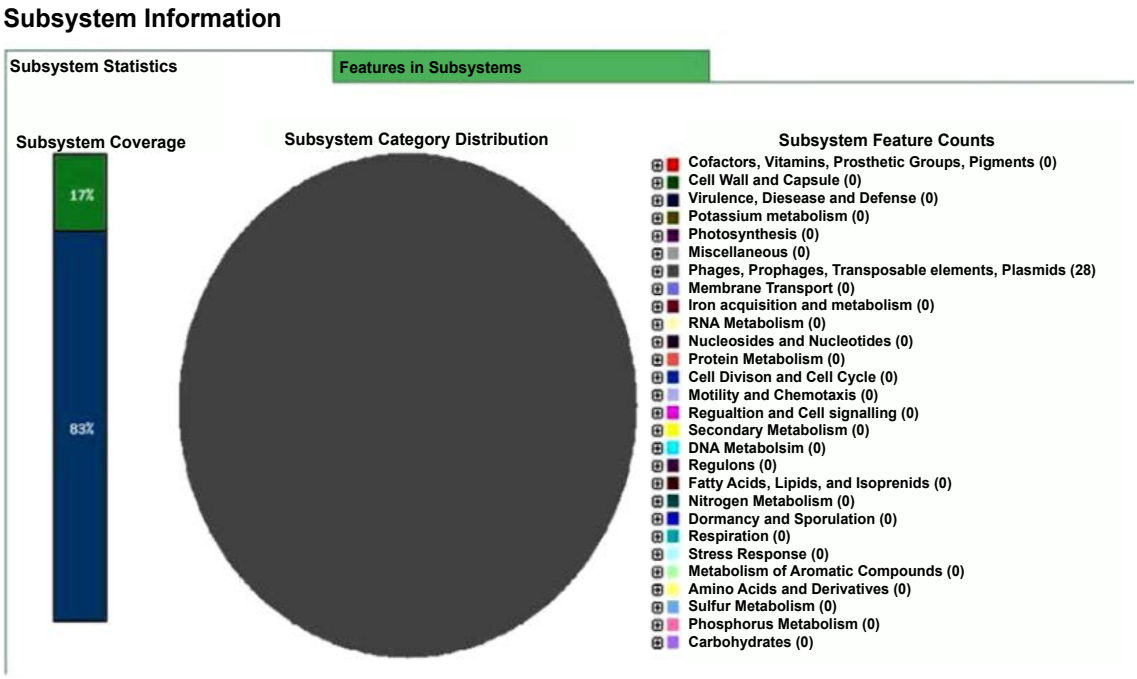


**Figure 2:** Sub system information of T1 Coliphage ADB-2.

[38]. As described in materials and methods, the 16S rDNA gene was amplified by universal primers. These sequences were blast at NCBI and submitted to NCBI. The detailed reports on each of the isolate studied for 16S rDNA shows aligned sequences of both culture studied for phylogenetic analysis. The sequence data were further analyzed for finding the closest homologs for the microbe by comparing gene sequence with reference strains. To analyze the phylogenetic position, the 16 S rDNA sequence of the strain ADB-2 (500 bp, JX094849), SMARJ-2 (542 bp, JX094848) were determined. Based on nucleotides homology and phylogenetic analysis the microbes were identified (Figure 1).

The data generated from the genomic library contained 229,781 reads and 45,496,800 nucleotide bases with average read length of 198 bases. The assembly using Newbler version 2.6 generated a 50,552-bp-long single chromosome.The phage has 46% GC with 76 predicted coding regions and 2 RNA genes. This genome contains functional genes related to phage structure andpackaging machinery like major capsid protein, unknown phage structure proteins, and terminase.

Phage neck protein, tail structure for host interaction like tail fiber protein, tail sheath protein, and tail-associated protein, phage DNA synthesis (helicase, DNA-directed RNA polymerases, endonuclease, and transcription regulator) and host lysis (endolysin without holing). These functional genes are scattered over the genome (Figure 2). The complete genome analysis of this phage provides new insight into its characteristics and interactions with *Escherichia coli.* Further comparative genomics would help us to understand the metabolic and adaptive complexity of such viruses.

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