1 • INTRODUCTION
1. Introduction
A virus is a very small, non-cellular parasite of cells. It’s DNA or RNA as their genetic material and is enclosed in a protein coat. Viruses are living being which is having capacity to infect all the cellular form. When viruses inserted their genome into their host these viral genome remnant in to host genome very long ago. The occurrence of viruses found at almost everywhere in soil, air and water. The diversity of viruses in aquatic environment is very high that infect the organisms that live in those environments. The study of many viruses is very important because it's directly concern with human health. Among all the known viruses, the majority viruses used human cell as their host cell and used to produce their progeny. The bacterium *Escherichia coli* found in human intestine have also been the subject of much study and many viruses have been found in this species. Only the few part of viruses has been discovered, major part will remain to be discovered. On the basis of Metagenomics study from natural environments the existence of many bacterial species that have not yet been cultivated and isolated in the laboratory; are also hosts to viruses. In the water of Ganges and Jamuna rivers of India Hankin (1896) noticed that so many tiny particles control the bacterial activity particularly *vibrio* spp. He concluded that this activity was filterable and destroyed by boiling. In addition, there is substantial literature on bacterial autolysis by Gamalieya, Malfitano, Kruse, and Pansini, which was reviewed by Otto and Munter (1923). At this point, however, it is difficult to provide unambiguous interpretations of these early studies. In initial experiment bacteriolysis observe on solid and liquid medium.

Are viruses living or nonliving?
Viruses are bridge between living and non living when they are outside the host behaves as a non living and after infection they have capable to express their gene, replicate and adapted inside the host. When viruses infect their host they used host's replication machinery to replicate their own gene in this way they consider as living. When viruses are outside their host cell they don’t reproduce and don’t give any other biochemical activity thus in this form considered as non living. You might form your own view as to whether viruses are living or nonliving after knowing more about viruses. After discovery of viruses the development in virology field makes it the huge subject. Now a days the term
virus also used in the field of computer to express the difficulties come in computer field.

**Figure 1.1:** Interaction between Virus molecule and cell molecule.

**Bacterial virus**

Viruses (bacteriophages) Bacterial viruses, known as bacteriophages or phages (from the Greek phagein, ‘to eat’), were discovered independently by Frederick Twort (1915) and Felix d’Herelle (1917). A diversity of phages has subsequently been identified and grouped into a number of families. Phage diversity is reflected in both morphological and genetic characteristics. The genome may be DNA or RNA, single- or double-stranded, circular or linear, and is generally present as a single copy. Morphology varies from simple, icosahedra and filamentous phages to more complex tailed phages with an icosahedra head. The majority of phages are tailed. Phages are common in most environments where bacteria are found and are important in regulating their abundance and distribution. The host controlled modification and restriction systems of bacteria are presumed to protect against phage infection: restriction is leveled against invading double-stranded phage DNA, whilst self-
DNA is protected by the modification system. However, in response, certain phages have evolved anti-restriction mechanisms to avoid degradation of their DNA by restriction systems. Broadly, phages can be classified as either virulent or temperate. A virulent phage subverts the cellular apparatus of its bacterial host for multiplication, typically culminating in cell lysis and release of progeny virions. In rare cases, for example the filamentous ssDNA phage M13, progeny are continuously extruded from the host without cell lysis. Accordingly, M13 has been referred to as a ‘chronically infecting’ phage. Temperate phages have alternative replication cycles: a productive, lytic infection or a reductive infection, in which the phage remains latent in the host, establishing lysogeny. The later generally occurs when environmental conditions are poor, allowing survival as a prophage in the host (which is referred to as a lysogen). During lysogeny the phage genome is repressed for lytic functions and often integrates into the bacterial chromosome, as is the case for phage lambda (λ), but it can exist extra chromosomally, for example phage P1. The prophage replicates along with the host and remains dormant until induction of the lytic cycle. This occurs under conditions that result in damage to the host DNA. The phage repressor is inactivated and the lytic process ensues. Such a mechanism allows propagation of phages when host survival is compromised. A resident prophage can protect the host from super infection by the same or similar strains of phages by repressing the incoming phage genome (a phenomenon known as super infection immunity). Some temperate phages contribute ‘lysogenic conversion genes’, for example diphtheria or cholera toxin genes, when they establish lysogeny, thereby converting the host to virulence. Phages can also mediate bacterial genome rearrangements and transfer non-viral genes horizontally by transduction. Tailed phages are the most efficient particles for horizontal (lateral) gene transfer, with the tail effectively guiding injection of DNA into the bacterial cell. Such phage activities generate variability and are a driving force for bacterial evolution. Bacteriophages have had key roles in developments in molecular biology and biotechnology. They have been used as model systems for animal and plant viruses, and have provided tools for understanding aspects of DNA replication and recombination, transcription, translation, gene regulation and so on. The first genomes to be sequenced were those of phages. Restriction enzymes were discovered following studies
on phage infection of different hosts and laid the basis for the development of
gene cloning. Phage-encoded enzymes and other products are exploited in
molecular biology. Certain phages have been adapted for use as cloning and
sequencing vectors and for phage display. Phages are utilized in the typing of
bacteria, in diagnostic systems, as biological tracers, as pollution indicators and
in food and hospital sanitation. There is also renewed interest in the therapeutic
potential of phages, due largely to the rapid emergence of antibiotic-resistant
bacteria and of new infectious diseases. However, realization of this potential
will depend on a number of factors including improved methods of large-scale
production and purification of phages, appropriate protocols for administering
phages and modification to enhance therapeutic properties, to remove phage-
encoded toxins, to avoid clearance of phages by the host defense system and
so on. This chapter considers the biology of RNA and DNA phages. Properties
and applications of a selection of phages that employ different strategies for
phage development will be discussed, with emphasis on tailless single-stranded
RNA and DNA phages.
Introduction

Figure 1.2: Time Line: Highlights in the development of phage as a potential therapeutic agent for bacterial infections

- 1915: Discovery of Phage
  - Sinclair Lewis “Arrowsmin” published
  - United States pharmaceutical companies market phage product
    - Eli Lilly market Staphylojel, E. R. Squibb & Sons page for staphylococcus and Abbott Laboratories combined page staphylococcus and colon bacillus

- 1925: D’Herrele uses phage therapy
  - avian typhosis in chickens, Shigella dysenteries in robots and bacillary dysentery in humans.

- 1926: D’Herrele established Tbilisi Phage Institute
  - Study on problems of commercial phage products

- 1930: Monson reports successful phage therapy for cholera epidemic

- 1932: Asheshov conducts experiments to determine if phage can effect experimental infections

- 1934: Monson reports successful phage therapy for cholera epidemic
  - Council on Pharmacy and Chemistry of the American Medical Association concluded that phage therapy was a questionable value

- 1937: Dubos rescues mice infected intracerebrally with Shigella dysenteries
  - Antibiotic overshadows phage therapy

- 1940: Monson reports successful phage therapy for cholera epidemic

- 1943: Council on Pharmacy and Chemistry of the American Medical Association concluded that phage therapy was a questionable value
Inchley shows that the reticuloendothelial system (mainly liver) removes intravenously injected phage.

Ochs and colleagues begin long series of studies using phage to probe human immune system.

Stent presents theoretical reasons for failure of phage therapy in Molecular Biology of Bacterial Viruses.

Geier shows oral phage infective for systemic distribution.

IET in wood began treating humans while phage therapy. Over the next 20 years, over 1,300 patients were treated.

Phage therapy for Vancomycin resistant enterococcus in mice.

Studies model pharmacokinetics of phage therapy.

Smith an Huggins perform several phage therapy experiments, including one that shows phage can more effective than antibiotics.

Bio tech industry begins exploring phage therapy in western countries.

Lederberg comments encourage phage therapy re-examination.

Mernil and colleagues develop therapeutically more effective long-circulating phage.

Phage therapy for methicillin resistant staph. aureus in mice.

Figure 1.2: Time Line: Highlights in the development of phage as a potential therapeutic agent for bacterial infection
Reasons for studying viruses

It was only later, however, after the pioneering work by Félix d'Herelle, that Twort’s report was recognized as dealing with bacteriophages (Twort, 1993). It may be living protoplasm that forms no definite individuals, or an enzyme with power of growth (Twort, 1915). After the discovery of Twort, Felix d'Herelle observed that certain antagonistic activity against bacteria was found in liquid as well as on solid medium. He observed clear zone of bacterial lysis and he called them plaque (d'Herelle, 1917). D'Herelle gives the term bacteriophage and described that these tiny microbes invade into the bacterial cell and multiplied. (Summers, 1999). In addition to his clinical duties, he pursued his research interest in the question of why enteric bacteria were sometimes pathogenic and sometimes not (summers, 1998), d'Herelle observed that in the viruses present in the filtrate of dysentery sample infect the pathogenic bacteria and alter their pathogenicity. To his surprise, he noted lysis in liquid culture and the formation of clear spots (later he called them teaches verges, “virgin spots”) in the conquest bacterial culture that covered the agar slants. Finally he gives the concept that invisible agent is the parasite of bacteria. His investigations extended and he noted that for the reproduction this invisible agent needed living cells, and that cell lysis seemed to be required for the multiplication process. After that D'Herelle concluded that plaque count is considering as the method for the enumeration of invisible agent. He was able to show that phage multiplied in waves, or steps, which he interpreted as representing cycles of infection, multiplication, release, and re-infection. D'Herelle pioneered two important areas of phage research. He reported that phage is the natural agent who gives the resistance against various bacterial diseases and can be used as a therapeutic agent in pre-antibiotic era. After discovery of phage Twort support to D'Herelle’s for their further research and observed biological characteristic of bacteriophage. When phage used for the treatment of dysentery and typhoid he observed that increasing the number of phage during the course of recovery. He concluded that the gradually lytic phages infect the pathogen for their multiplication and that was the actual mechanism of recovery. He called phages “exogenous agents of immunity (D'Herelle, 1917, p. 375).”
Some viruses cause disease

Viruses are the causative agent for the human disease some viruses cause common cold while some are more lethal e.g. rabies and viruses also responsible in the development of several types of cancer. The viral disease gives great impact on society in early day’s small pox and in this time AIDS. The disease. This shows the requirement to study how they are infected and caused disease. For the prevention, diagnosis and treatment of virus diseases the knowledge of viruses helps and also used for the production of vaccines, diagnostic reagents and techniques, and antiviral drugs. The use of viruses for the medical concept particularly in veterinary and in agriculture field is very important because of the economic impact in this field. In dairy product like cheese, yogurt and other product in which lactic acid bacteria used as a starter culture for the fermentation process where viruses can cause economic damage.

Some viruses are useful

- **Phage typing of bacteria:** Some species of bacteria can be classified on the basis of their susceptibility towards phage. During the outbreak of disease phage typing can also be useful for the identification of pathogenic bacteria.

- **Sources of enzymes:** The enzymes reverse transcriptase and RNA polymerase used in the field of molecular biology can be obtain from viruses.

- **Pesticides:** Some insect pests commonly found in agriculture field are controlled with viruses.

- **Anti-bacterial agents:** In the pre antibiotic era scientists realized the therapeutic value of phage due to their anti bacterial activity again in mid-20th century phages were used to treat some bacterial infections with the emergence of antibiotic-resistant strains of bacteria.

- **Anti-cancer agents:** Some genetically modified strains of viruses are being useful for the diagnosis as well as for the treatment of cancers and tumor.
• **Vector:** Gene vectors for protein obtain the product of some gene and for the vaccine production require cloning technique in which viruses used as a vector for the desire gene.

• **Treatment:** Some viruses are used for treatment of genetic diseases.

**Phages of Gram-Negative Bacteria**

• **Lambda** a 48,502-bp siphovirus is the best-studied of the temperate coliphages; It was one of the first phages sequenced (Sanger et al., 1982), and the intensely explored lambdoid phage group has strongly influenced our view of phage evolution.

• **Mu** is polyvalent bacteriophage used *E. coli*, Salmonella, Citrobacter, and Erwinia as their host cell.

• **N4** is the phage which contains 72-kbp genome and it is podoviridae group of virus which used *E. coli K-12* as their host. This is phage having the DNA dependent RNA polymerase. Also used as a modal organism to study of transcriptional regulation.

• **N15** is one type of lysogenic phage and also considers a member of a coliphage.

• **P1** is a well-studied temperate myovirus of enteric bacteria that infects a broad range of gram-negative hosts. P1 and its sister, *P7*, are unusual in that the prophage most commonly exists free as a plasmid rather than integrated into the host chromosome. It is particularly useful because it can carry out generalized transduction of genes between strains of *E. coli* and Shigella.

• **P2 and P4** are generally considered as a pair because P4 has no genes for structural proteins of its own. Rather, it has the ability to instruct the main head protein of P2 and related temperate phages to assemble into a particle one-third the normal size the right size for the P4 genome (11,624 bp) instead of for the P2 genome (33,593 bp).

• **P22**, a generalized transducing phage of *Salmonella typhimurium*, was the phage involved in the initial discovery of transduction by Zinder and Lederberg (1952). P22 is a member of the Podoviridae. The 41,724-bp genome includes 64 genes and 52 Bacteriophages: Biology and
Applications unidentified ORFs. Its genes are clustered by function in the same general order as in lambda, with which it can exchange certain blocks of nonstructural genes. In P22, as in many other phages, the DNA is packaged by head starting from a specific pac site and proceeding unidirectional along a long replicative DNA concatamer, with packaging of the next pro-head then starting wherever the previous one finishes. Generalized transduction is thought to be a consequence of the occasional packaging of host DNA starting at some pac-like site and continuing through head of bacterial DNA. The P22 DNA packaging apparatus and pac site have been used very effectively in building cloning vectors, as discussed in Chapter 11. Upon entry into the host, the DNA circularizes and then either integrates into a specific chromosomal site to form a pro-phages or replicates via a rolling-circle process to form a new concatamer. P22 can be very advantageous to its host. In addition to exclusion of phages in the same immunity group through the repressor involved in maintaining lysogeny, P22 pro-phages express genes that interfere with DNA injection by related phages, that alter the O-antigen structure to interfere with further P22 adsorption, and that abort the lytic cycle of some other Salmonella phages.

• ΦKZ is the best-studied member of a broad and distinct worldwide family of giant Pseudomonas phages with 120-nm isometric capsids and contractile 200 nm tails.

• T1 is a virulent siphovirus belonging to the original set of seven T-phages infecting E. coli B chosen by Delbrück for use in the early development of molecular biology. It is best known for remaining viable even when it dries out. It can therefore wreak havoc on a microbiology laboratory if it is once brought in, and relatively few studies have been done with it. Its 50.7-kbp circularly permuted, terminally redundant genome has recently been sequenced and 77 ORFs identified, including many small proteins that show no homology with known phage or other proteins (Andrew Kropinski, personal communication). The tail genes show homology to those of N15, but the head genes are unique.
• **T4**, another classic coliphage, is a 169 - kbp lytic myovirus that substitutes 5- hydroxyl methyl cytidine (hmdC) for cytidine in its DNA. It played a central role in the development of molecular biology and is one of the most thoroughly studied biological entities on earth (along with its close relatives T2 and T6—the T-even phages). T4-like phages are prime components of a number of therapeutic cocktails and several other members of its broad family, infecting a range of gram-negative bacteria, have recently been sequenced.

• **T5** is a siphovirus from the original set of T-phages.

• **T7** and its close relative T3 are the final two members of the original T-phage set of coliphages; similar 40-kbp podoviruses are found infecting virtually all gram negative bacteria, and individual broad host range variants have been found that can, for example, infect both *E. coli* and *Yersinia pestis*.

### Phages of Gram-Positive Bacteria

• **C1**, one of the first phages isolated (Clark, 1926), is a lytic 16,687-bp podovirus infecting group C streptococci that was involved in the early development of phage typing (Evans, 1936).

• **G** is an enormous lytic myovirus that infects *B. megaterium* the largest characterized virus, with more genetic information than many bacteria. MM1, a siphovirus with a 40,248-bp genome, is the first temperate *Streptococcus pneumoniae* phage to be sequenced (Obregon *et al.*, 2003).

• **Φ11**, the best studied of the temperate phages of *Staphylococcus aureus*, is a transducing siphovirus.

• **Φ29** is a rather small virulent phage (19,285 bp) infecting *B. subtilis*.

• **Φ105 and SPb** are the most-used transducing phages for *B. subtilis*.

• **Sb-1** is a virulent phage infecting *S. aureus*. Staphylococcal infections are among the major infections susceptible to phage treatment, and Sb-1 and its relatives are major components of the Eliava Institute pyophage therapeutic phage preparations against purulent infections, as well as being effectively used in monophage formulations. This myovirus is the only
phage that has been used intravenously for therapeutic applications in the Republic of Georgia

- **SPO1** and its relatives are large, virulent *B. subtilis* myoviruses

**Application of Phage Therapy**
- Biofilm infection control
- Treatment of mycobacterium infections
- Alteration of host binding profile
- Use of bacteriophage in SASP technology
- Epidemiological fingerprinting of bacteria isolates (Phage typing)
- Use of phages as tools in molecular biology
- Use of bacteriophage to express peptides and proteins (Phage display)
- New phage diagnostic tools
- Important ecological role in recycling or organic matter including cells
- Use of bacteriophage in the preparation of Engineered prophage

**Classification of Phage Therapy**

Phage therapies can be classified into five categories in terms of the likelihood and nature of human contact. They are:

- **Category I**: Human exposure to the environment to which phages have been applied is unlikely and therefore human exposure to the applied phages is rare;
- **Category II**: Human exposure to the environment is likely, but human exposure to applied phages is greatly reduced;
- **Category III**: Human exposure to the environment is likely and human exposure to phages is somewhat likely;
- **Category IV**: Phages are directly applied to humans, but without deliberate introduction of phages deeply into human tissues;
- **Category V**: Phages are deliberately introduced deeply into human tissues.
Introduction

**Need of Indicator & Biocontrol Agent of Coliform in Poultry**

Salmonellosis is the major issue associated with human health throughout worldwide. It has been estimated that 13 million case of salmonella infection found in worldwide per year among all these cases 70% occurs from China, India, and Pakistan (Gupta and Verma, 1993). The U.S. Centers for Disease Control and Prevention report approximately 40,000 culture confirmed cases of salmonellosis each year in the United States, which result in approximately 400 deaths (Bertani et al., 1953). Salmonella infection is very common problem associated with meat and poultry. Poultry fecal material is the best natural medium for the growth of salmonella, during the processing salmonella come in contact with animal. (Centers for Disease Control and Prevention. 2005; European Food Safety Authority, 2004; Velge et al., 2005.). It was reported that in European Union (EU) S. enteritidis and S. typhimurium infection in human is, respectively, 76% and 14%. (European Food Safety Authority, 2004; Fiorentin, et al., 2005.). Because of above reason it is necessary to set the goal to control the salmonella infection in poultry industry (Atterbury et al., 2007; Bielke, et al., 2007; European Food Safety Authority, 2004; Velge, et al. 2005). The excess use of antibiotic and non medical use of antibiotic in both humans and animal increase the problem of resistance in pathogenic strain of bacteria and makes less and less effective antimicrobial activity. (Arlet et al.; 2006). Thus, the problem of antibiotic resistance shows the need to development of alternatives of chemotherapy in critical priority. The use of bacteriophages, viruses that specifically infect and lyse bacteria, as a therapeutic agent (phage therapy) is one possible option for controlling pathogenic bacteria. Phages also used as an indicator as well as biocontrol agent for salmonella in human and animal and also noticed that somany advantages over the use of antibiotic. (Barrow et al. 2007; Matsuzaki. et al 1997; Petty et al. 2007; Skurnik et al. 2007). However, Host specificity of phage makes it very target specific and can be used only against their natural host (Bielke et al., 2007 ), which, in the case of pathogenic hosts when it lysed it produced cell debris and large quantities of both endotoxins and exotoxins (Clark et al., 2006; Skurnik et al., 2006; Tinsley et al., 2006). After the discovery of phage purification technique makes the easy way for the phage preparation leading to easy and faster approval of phage
products. But to find the multivalent phage is still considered as a very difficult task. (Bielke et al., 2007; Krylov et al., 2006; Skurnik et al., 2006). The cost of Medical treatment against salmonella infection is considerable in many countries. Total 2500 salmonella serovar distributed in this world among all the species some of host specific for example S. typhi, S. gallinarum, S. dublin and S. choleraesuis while remaining are host non specific but capable to cause the disease in human and animal particularly chickens, are known to be the main reservoir for this infectious agent. (Ellner, 1978; Murugkar et al., 2005). The primary goal of this research work is to isolate and characterized a lytic coliphage with a broad host range.

After some attempt it make possible to control the salmonella infection in poultry industry by use various antimicrobial an improvement in biosecurity but still it remain major problem. With the help of host-specific bacteriophages as a biocontrol is one possible alternative to reduce the salmonella colonization. During the year of 2004-05 more than 200 bacteriophages were isolated which was having the capacity to infect salmonella serovars from various sources. Poultry is known to be the major single reservoir of Salmonella. The causative agent of fowl typhoid is S. gallinarum, is the most prevalent host-adapted Salmonella strain in India (Gupta et al., 1999). S. typhimurium and S. enteritidis not only involved in severe outbreaks of avian salmonellosis and economic losses to the poultry industry (Verma and Gupta, 1997; Palaniswamy et al., 1989), these pathogens also responsible to transmit the disease from poultry to human (Rahman et al., 1997). The developments in bio-security of poultry farms are likely to be more expensive and very difficult to maintain Davies, 2005), so this shows a need to find an acceptable, cost effective way of preventing the incidence of Salmonellosis in poultry (Atterbury, 2006). Use of bacteriophage is one possible method to control the outbreak of zoonotic pathogens in poultry in recent years (Sulakvelidze et al., 2001; summers, 2001) Because of the capacity to infect the bacteria, bacteriophage is consider as a natural predators of bacteria and are ubiquitous in the environment (Rohwer and Edwards, 2002). The poultry industry is a significant economic force in the World; the demand for chicken has increased day by day. (http://usda.mannlib.cornell.edu/reports/nassr/poultry/ppy-bb; accessed 9 May 2006). E. coli is also considering as the causative agent of cellulitis, septicemia, and
air sacculitis which is communally found in poultry; therefore, it is also require equal attention as required on other bacterial pathogen of broiler chickens. Tetracycline and streptomycin both is approved antimicrobial agent to control the *E. coli* infection in broiler chickens (Poppe, C., 1999). But the problem of cost and resistance still associated with the use of these antimicrobial agents (Atterbury *et al.*, 2007). The use of fluoroquinolones, sarafloxacin and enrofloxacin for the treatment of *E. coli* infections in poultry is restricted by the U.S. Food and Drug Administration. The transfer of antimicrobial resistance genes to pathogenic bacteria is the major problem in farm environments (Matsuzaki *et al.*, 2005, Tinsley *et al.*, 2007). Since bacteria acquire most resistance genes through horizontal transfer, conjugation of genetic elements such as plasmids and transposons are common vectors for of antimicrobial resistance genes in various microorganisms.
OBJECTIVES

- Isolation, enrichment and purification of bacteriophages
- Determination of polyvalence (host range) of isolated bacteriophage
- Determination of antibiogram of natural host strain
- Identification of natural host strains
- Isolation, purification and bacteriophage DNA
  I. Determination of molecular weight
  II. Detection of specific genes
  III. Detection of mutation in specific genes
  IV. Whole genome sequencing of bacteriophage DNA