ISOLATION AND CHARACTERIZATION OF POSSIBLE NEW BACTERIOPHAGE FROM GOAT FAECES

by

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LIST OF ABBREVIATIONS

APS	Ammonium sulfate phosphate
bp	base pair
$CaCl_2$	Calcium chloride
ddH_2O	Deionized water
DNA	Deoxyribonucleic acid
DNase 1	Deoxyribonuclease
dATP	Deoxyadenosine triphosphate
dNTP	Deoxyribonucleotide triphosphate
E.coli	*Escherichia coli*
ETBR	Ethidium bromide
EDTA	Ethylene diaminetetraacetic acid
E.M.	Electron Microscope
H_2O	Water
ICTV	International Community Taxonomy of Viruses
kbp	Kilobase pair
LB	Luria Bertani
$MgCl_2$	Magnesium Chloride
NaCl	Sodium chloride
NCBI	National Centre of Biotechnology Information

nm	Nanometer
PCR	Polymerase Chain Reaction
PEG-6000	Polyethylene Glycol 6000
Pfu	Plaque forming unit
RE	Restriction endonuclease
RNA	Ribonucleic acid
RNase A	Ribonuclease A
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
Taq	*Thermos aquacticus*
Tris-base	Tris (hydroxymethyl)-aminomethane
T4 PNK	T4 Polynucleotide Kinase
TBE	Tris/Borate/EDTA
TEMED	Tetramethylethylenediamine
T.E.M	Transmission Electron Microscope
v/v	Volume/volume
w/v	Weight/volume

LIST OF APPENDICES

PEMENCILAN DAN PENCIRIAN BAKTERIOFAJ BERKEMUNGKINAN PENEMUAN BARU DARIPADA TINJA KAMBING

ABSTRAK

Bakteriofaj atau faj adalah kumpulan virus yang mempunyai kebolehan menjangkiti dan melilis bakteria. Faj adalah salah satu daripada benda hidup yang terbanyak di dunia dengan anggaran bilangan 10^{31} faj. Setakat ini, hanya ~6,000 faj telah ditemui dan kebanyakan adalah faj DNA berbenang dua dan berekor. Penggunaan faj sebagai rawatan terhadap jangkitan bakteria telah mendapat perhatian semula disebabkan oleh kemunculan strain bakteria rintangan pelbagai antibiotik. Tambahan pula, kepelbagaian faj dengan jumlah yang besar juga menyumbang kepada terapi faj. Satu faj berkemungkinan baru telah berjaya dipencilkan dari tinja kambing yang boleh menambah kepada jumlah faj yang ditemui sehingga kini dan memungkinkan penggunaannya dalam terapi faj. Faj yang dipencilkan adalah DNA berbenang dua dan berekor dengan morfologi serupa seperti faj yang lain tetapi mempunyai struktur ekor yang unik. Faj mempunyai struktur ekor yang kaku tetapi faj yang dipencilkan ini mempunyai struktur ekor yang fleksibel. Di samping itu, faj ini mempunyai kapsid berukuran 50-57 nm pada ukur lilitnya dan ekor berukuran 107 nm panjang serta genom DNA ganda dua. Berdasarkan ciri-ciri yang ada pada faj yang dipencilkan ini, ia berkemungkinan tergolong dalam famili *Siphoviridae*. Faj ini menunjukkan sifat fizikokimia yang agak berbeza apabila dibandingkan dengan faj berekor yang lain. Ia mempunyai lingkungan suhu dan pH kemandirian yang lebih luas berbanding dengan faj biasa seperti T4, dengan suhu 70^0C dan pH 5-11. Analisa

proteomik dan genomik juga menunjukkan perbezaan apabila dibandingkan dengan faj biasa. Saiz genom untuk faj yang dipencilkan adalah dianggarkan dalam linkungan 30-40kbp. Berdasarkan kepada hasil kajian morfologi, fizikokimia, proteomik dan genomik, faj yang telah berjaya dipencilkan ini berkemungkinan merupakan tambahan virus baru di dalam pangkalan data Jawatankuasa Antarabangsa Taksonomi Virus (ICTV).

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ABSTRACT

Bacteriophages or phages are group of viruses that would infect and lyse bacteria. Phages entities are one of the most abundant life form on earth with the total estimation number of 10^{31} phages. Until today, only ~6,000 types of phages have been discovered and majority of them are dsDNA tailed phages. The use of phages as a treatment for bacterial infections has regained the attention due to the emergence of antibiotic multi-resistant bacterial strains. In addition, the diverse and enormous phages also contribute to the bacteriophage therapy. A potentially new bacteriophage was isolated from goat feces which could add to the number of phages discovered so far and could be used in phage therapy. The isolated phage is a tail phage with similar morphology to other tail phages, yet it has unique tail structure. Other phages have stiff tail structures but this isolated phage has a very flexible tail structure. Besides that, the newly isolated phage has capsid measures 50-57nm in diameter and tail measures 107nm in length with double stranded DNA genome. These characteristics make the isolated phage could be grouped into the family of *Siphoviridae*. The isolated phage shows quite different physiochemical characteristics as compared to a common tailed phage. The isolated phage has wide range of temperature and pH tolerance for its survival compared to other tail phage such as T4, with temperature of 70^0C and pH 5-11. Proteomic and genomic analysis

show differences between the isolated phage when compared to common phage. Genome size estimation of the isolated phage is around 30kbp – 40kbp. Based on the morphology, physiochemical, proteomic and genomic analysis results, the isolated phage could be a new addition to the list of viruses in International Committee on Taxonomy of Viruses (ICTV) database.

1.0 INTRODUCTION

Based on the International Committee on Taxonomy of Viruses (ICTV) report, viruses are the most abundant life form on the earth and play major roles in the ecological balance of the microbial life forms (Forest, 2003; Pedulla, *et al.*, 2003). Viruses could be found almost everywhere, in fact wherever cellular life could be found, viruses also could be found (Ackermann, 2001).One of them is bacteriophages or phages for short which refer to group of viruses that infect bacteria as their hosts. It is estimated that there are approximately 10^{31} types of phages in the biosphere (Wommack and Colwell, 2000).

Generally, phages could be found everywhere, in environmental ecosystem as well as in gastrointestinal ruminants' that lead them to be the most diverse microorganism. Gastrointestinal ruminants' is a favorable habitat for most of the microorganisms such as bacteria, archaea, fungi, bacteriophage and protozoa. Huge populations of bacteriophages could be found in gastrointestinal ruminants' since it provides suitable environment and their hosts are plenty in numbers. It is estimated the free bacteriophage particles in gastrointestinal of ruminant is about $10^8 – 10^{11}$ per milliliter (Indira, 1999 and Roderick, 2002). In this study goat feces was selected because goat is also under the category of ruminant and so far phages isolated from goat feces are low in number. Therefore, with an assumption there might be number of new bacteriophage could be isolated from goat feces, this sample was chosen.

The fact is that, in this abundant diversity, so far only ~ 6,000 types of phages have been discovered since 1959 and 96% of them are tailed phages (Ackermann, 2001). This finding showed that there are a lot more phages waiting to be discovered in each day. Besides that, bacteriophages have many beneficial applications not only in the field of medicine but also in agriculture, fishery and environment. (Inal, 2003).

ICTV is a committee which authorized and organized the taxonomic classification of viruses. This committee has been given the full governing authority in term of viruses' classification according to the universal taxonomic scheme. The list of known and discovered viruses details are up dated regularly by this committee and the latest update was March 26, 2011. According to this update there are 6 orders, 87 families, 19 subfamilies, 348 genera / genus and 2285 species have been discovered and registered under ICTV (ICTV report, 2011). These numbers are the total count of animal, plant and bacteria viruses. The taxonomic database details showed that the number of bacteriophages is less compared to plant and animal viruses.

For phages, there is one order with 3 families, 2 subfamilies, 23 genus and 57 species have been registered so far (ICTV report, 2011). ICTV taxonomic database also identified 60 families of viruses which not assigned to any order. In these 65 families of viruses, 10 families are phages that have not been assigned to any order. (Ackermann, 2003 and Daniel, 2004). Therefore, without doubt there are many more phages waiting to be discovered and classified. Far these, the list in ICTV database are growing by the days.

Malaysia is one of the countries that loaded with mega biodiversity of not only flora and fauna but microbial communities are as well (Ministry of Natural

Resources and Environment, 2006). The fact is that current focus in mega diversity is only on flora and fauna while the microbial communities are left undisturbed. But the reality is that the microbial community is closely related to flora and fauna that cannot be separated for the existence of cellular life (Greenpeace International Report, 2004). Taking advantages of Malaysia being a mega biodiversity, then the phages communities would be much diverse as well. The general view of this thesis project is to isolate and characterize novel phage.

The work present in this thesis could contribute to the growing list of phages in ICTV database since there are still a lot of phages waiting to be discovered. Thus, the objectives of this work are:

I) To isolate novel bacteriophage from gastrointestinal of goat (through feces) since it is one of the primary reservoirs for phages

II) To characterize the isolated phage based on:

(a) morphological features (size, diameter and shape)
(b) physiochemical attributes (pH, temperature stability and phage susceptibility)
(c) molecular aspects of genomic
(d) proteomic studies.

2.0 LITERATURE REVIEW

2.1 Introduction to viruses

The word virus originally referred to any poisonous thing, such as the venom of snake. In Latin, the word virus means poisonous fluid (Kathleen, 1999). In biological term virus is a simple, acellular entities consisting of one or more molecules of either DNA or RNA enclosed in a coat of protein. (Prescott, *et al.,* 1999). Furthermore, viruses also referred to as subcellular organism, which means they are smaller than most of the cells even in comparison with bacteria and human cells (Figure 2.1). The average sizes of viruses are measured in nanometer (nm) while bacteria are measured in micrometer (um) (Prescott, *et al.,* 1999). It means viruses are thousand times smaller than bacteria. Therefore, viruses are not able to be viewed with naked eye or even normal microscope. The only way to view them is by using the most powerful electron microscope (Figure 2.2). Among viruses the mimivirus virus is one of the largest viruses which are about 400nm in diameter (Xiao, *et al.,* 2009) and the smallest virus, polio virus is only 28nm in diameter (Summer, 2001).

Viruses have the capability to enter the host cells via various routes such as ingestion, inhalation and direct contact. Once they gain the entrance, they would use host cells to manufacture substances needed for their own replication and life cycle (Harrison, 2001). They are only active when inside a host cell. Even though they are known to be the most powerful causative agent but they are not able to make own protein, energy and reproduce without a host cells. Therefore, outside a host virus are in dormant stages where they exist in a world between the living and non-living (Harrison, 2001; Clark and March, 2006).

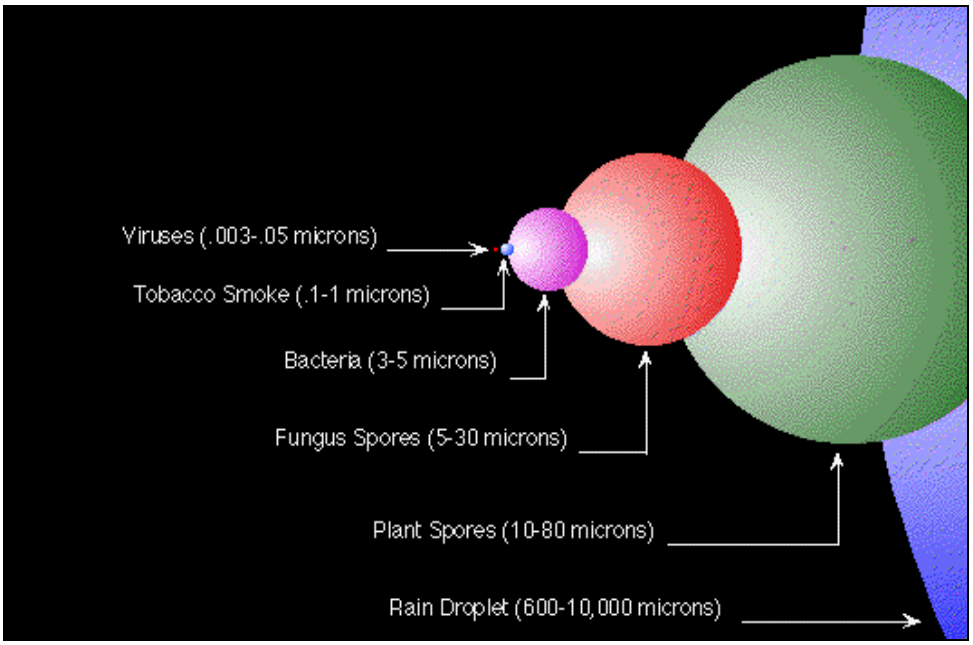


Figure 2.1: Size comparison between viruses and various small particles. (Adapted from Washington, et al., (1997). Koneman's Colour Atlas and Textbook of Diagnostic Microbiology 5th Edition)

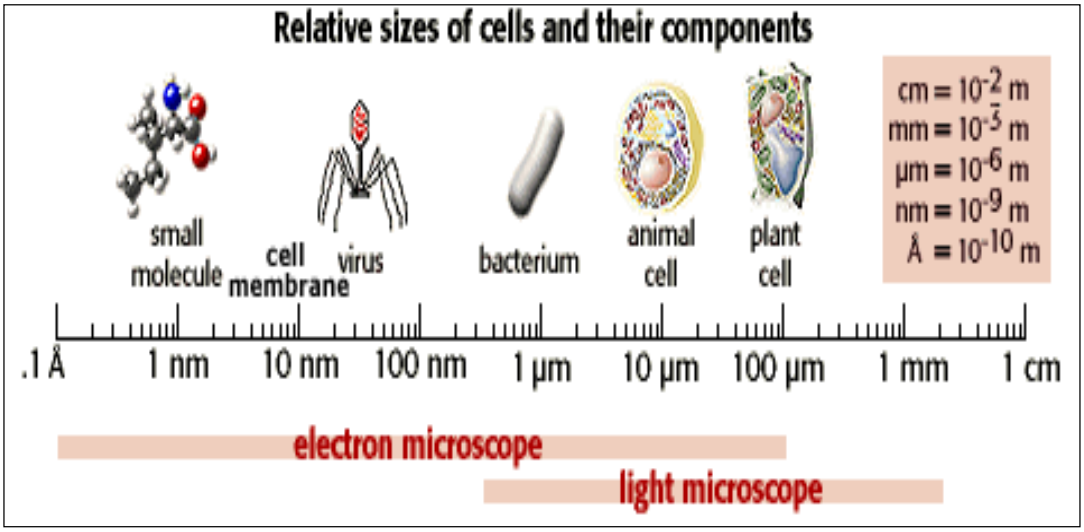


Figure 2.2: Measuring units and equipments used to make comparison between viruses and other organisms. (Adapted from Holt, et al., (2002). Textbook: HRW Modern Biology)

The most common feature of viruses is their shapes which could be used to distinguish them into few groups. Based on this feature, viruses cab be classified into two groups and it is also known as symmetry classification. They can be either helical or icosahedral in shape (Raven, *et al.,* 2005). In general, the helical symmetry viruses are rod in shape. The length of helical viruses are determined by the length of nucleic acid while their width is determined based on their size and packaging of their protein subunits. An example of a virus with helical shape is the tobacco mosaic virus (TMV) shown in Figure 2.3 (Zaitlin, 1999; William and David, 2002).

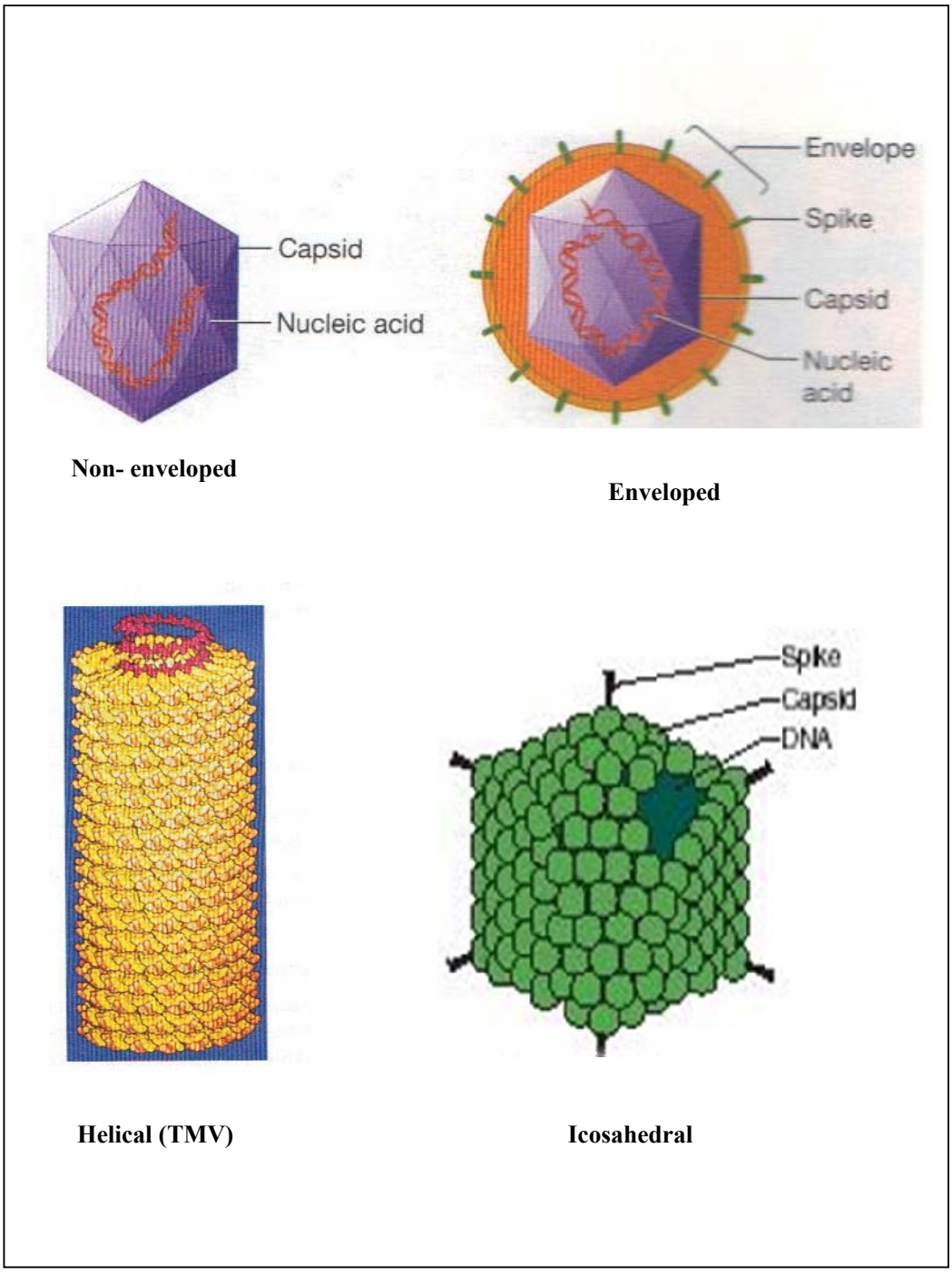
The other shape of virus is icosahedral which is different from the helical shaped viruses. A symmetric structure of an icosahedral showed it has 20 faces and roughly spherical in shape (Baker, *et al.,* 1999). In comparison with helical symmetry, the icosahedral symmetry is the most efficient arrangement for subunits in closed shell because it uses the smallest number of units to build a shell. While in helical symmetry, larger numbers of protein subunits arranged in a helix form (Baker, *et al.,* 1999; William and David, 2002).This classification is only based on the ordinary structure or shape of the viruses.

Viruses could be further classified based on the presence or absence of an envelope. Generally, viruses consist of capsid, either helical or icosahedral and nucleic acid but some viruses have extra feature known as an envelope (Baker, *et al.,* 1999).These viruses are called as enveloped viruses where viruses without an envelope known as naked virus. In general, the envelope consists of lipid bilayer with proteins (glycoprotein) embedded in it. Enveloped viruses are common in

animal world such as Influenza virus (Harrison, 2001; William and David, 2002). Figure 2.3 shows the differences in shape between helical, icosahedral, an enveloped and a naked virus.

What is a virion? A single virus is called virion that consists of a nucleic acid genome where the virus's heredity information is stored. It is basically surrounded by a protective shell of protein called a capsid (Raven, *et al.,* 2005). Instead of cell wall membrane, some viruses have capsid made of protein spikes which help them to penetrate into the host cells. Besides, the capsid protects the nucleic acid genomes containing the virus' genetic material (Summer, 2001 and Raven, *et al.,* 2005).

Generally, viruses consists either DNA or RNA and these genetic material required for their cell to reproduce. The nucleic acid can be either double stranded or single stranded (linear or circular) depending on the virus. Even though virion consists of nucleic acid genomes but in extracellular state the virion is metabolically inert and does not carry out respiratory or biosynthetic functions. Basically, they are not cells and they do not have cytoplasm, cellular organelles, and nucleus and cannot perform metabolism on their own (Ackermann and Dubow, 1987; Summer, 2001; William and David, 2002). However, just by carrying their genetic material viruses are able to survive within the biodiversity in the world. For all these characteristics, uncertainties of whether viruses are living or non-living are still vague or debatable.



Non- enveloped

Enveloped

Helical (TMV)

Icosahedral

Figure 2.3: Different morphologies of viruses. Viruses could be icosahedral or helical, as well as enveloped and non-enveloped. (Adapted from Joanne, M.W. *et al*., (2008). Microbiology. 7th Edition)

2.2 History of viruses

Viruses had enormous impact on human and other organisms but their nature and biological information was very little (Katleen, 1999). A brief history of their discovery and recognition as uniquely different infectious agents can help clarify their nature. The study of virology has been started in 19th century. The French bacteriologist, Sir Louis Pasteur revealed that rabies was caused by a "living things" which smaller in size than bacteria. In 1884, he was able to develop vaccine for rabies. Besides, he also proposed the term virus to classify the unique groups of infectious agents (Prescott, *et al.,* 1999 and Raven, *et al.,* 2005). Following this, the curiosity within the researchers about these infectious agents made them to do further work and contribute to the development of virology field.

At very first in 1892, Sir Dmitri Iwanowsk a Russian botanist found an agent which smaller than bacteria in size which able to pass through ceramic filter that enough to trap all bacteria. The development of porcelain bacterial filter by Sir Charles Chamberland in 1884, made possible the discovery of what are now called as viruses (Kathleen, 1999). Tobacco mosaic disease, that caused by virus was the first to be studied with Chamberland's filter. Later in 1898, Sir Martinus Beijernick confirmed Iwanowski's finding about the tobacco mosaic virus is something new. He named the finding as "*contagium vivum fluidum*" which means soluble living germ (Zaitlin,1999).In the same year Sir Paul Frosch and his partner Sir Friedrich Loeffler observed a similar agent was responsible for mouth and foot diseases rather than by a toxin (Williams and David, 2002 and Ackermann, 2003).

As years goes, study was carried out in finding the newly discovered agent. After years, in 1900 Sir Walter Reed found that yellow fever that thought to be mosquito borne infection was actually caused by a virus. Thus by the beginning of 20th century, it had been established that filterable viruses were different from bacteria and capable to cause disease in humans as well as plants. It was soon discovered that bacteria themselves also could be attacked by virus. Further in 1935, the chemical nature of viruses was revealed when in 1935, Wendell M. Stanley managed to crystallize the tobacco mosaic virus (TMV). He found that it to be a completely protein (Zaitlin, 1999). Lately, it was clearly proven that viruses are complexes of nucleic acids and proteins that able to reproduce only in living cells which will be their hosts (Williams, 2002).

2.3 Virus life cycle

2.3.1 Steps in virus life cycle in order to produce new virus particles

Each microorganism has its own life cycle and same goes to virus. There are 7 steps in the viral life cycle which are attachment (adsorption), penetration, uncoating, replication of viral DNA, assembly of viral particles, maturation and release of new viral particles (Ackermann and Dubow, 1987; Summer, 2001). Figure 2.4 shows the 7 steps taken in viral life cycle.

The 1st step is **attachment** where the virus will contact with it's specific host and it will attach at the specific spot on the cell surface (plasma membrane). It is a process of specific binding between viral capsid proteins and specific receptors on the host cellular surface (Summer, 2001).These receptors can be either protein component or other component that are located only on certain cells. This specificity determines

the host range of a virus. Basically, this mechanism has evolved to favour those viruses to infect only cells in which they are capable of replication (Martin, 2009).

After the attachment, the 2nd step is **entry or penetration,** followed by attachment**.** In general, viruses with lipid envelope the penetration of nucleo capsid core of a virion into a cell occurs when the virion envelope starts to fuse with the plasma membrane once it attached (Edward, 2006). Virion enters the host cell by membrane fusion. The fusion of viral cellular lipid membrane is usually mediated by proteins encoded by the information found in the viral particle. The penetration of virion is also depends upon their hosts. Basically, plants have rigid cell wall made of cellulose and fungi made of chitin. In these phenomena, viruses will release chemicals in order to weaken their host cell wall (Ackermann and Dubow, 1987; Wonmack and Colwell, 2000).Completion of this step called as viral entry.

Once the virion managed to enter the host, the following 3rd step will be **uncoating**. In this process there is need of viral enzymes or host enzyme in order to remove the viral capsid. Once the capsid is removed the viral nucleic acid will accessible to the cellular environment (Greber, *et al.,*1994). The 4th step is **viral genome replication**, one of the most important steps in viral life cycle. When a viral genome either DNA or RNA enters cellular environment they have two targets to complete. They have to express the new viral protein in temporal order and replication of the nucleic acid. This replication step is totally depends upon their nucleic acids (DNA or RNA) (Wonmack and Colwell, 2000). Whereby, the viral will control the host's machinery and direct the host to replicate the viral genome (Juliet, *et al.,* 1998).

After the replication process, the 5th step, **assembly of viral particles** will take place. In this part, the host cell will help to assemble new viruses until the host cell is full with new viral particles. It involves the assembly of all the components necessary for formation of mature virion at particular site. The assembly process occurs at different site depending upon the type of viruses. Generally, it can be either at nucleus, cytoplasm or inner surface of the cell membrane (Juliet, *et al.,* 1998; Jason and Edwin, 2001).

After the assembly of new virus particle, the following step is **virion maturation**. This is the 6th step in virus life cycle. At this point, there will be structural changes in viral particles. This is to allow the virus to be equipped for the next infection stage (Garoff, *et al.,*1998) In some viruses, the assembly and maturation of virion inseparable process while in certain viruses both are different mechanisms (Greber, *et al.,*1994; Jason and Edwin, 2001).

The final stage, the 7th step is **releasing** of newly formed viral particles. The releasing process is dissimilar between the enveloped and non-enveloped viruses. The enveloped viruses release from the host cell by budding (Jason and Edwin, 2001). They acquire lipid membrane as the buds out through the cell membrane. Where else in most non-enveloped viruses the final process is simple, in which the cells break open and release the viruses. (Edward, 2006) The new viral particles will be release through this spot, it will infect the new host cells and the cycle is repeated.

2.3.2 The assembly of pre-formed viral components

In the seven stages of virus life cycle as mentioned above, assembly of preformed components of virions is a vital stage in each and every virus life cycle. Generally, in double-stranded DNA viruses, the capsid subunits initially form a precursor capsid that is packaged with DNA. Later, it will subsequently mature as infectious viral particles. For example, this process will occur in bacteriophages, herpesviruses and adenoviruses (Raven, *et al.,* 2005). For the assembly of progeny virion, it requires additional proteins termed scaffolding proteins that not present in the mature capsid (Juliet, *et al.,* 1998).

However, the site of assembly and morphogenesis is depends upon the type of viruses. Usually, with DNA viruses the capsid assembly will occur in nucleus except for hepadnaviruses and poxviruses where the assembly will happen in cytoplasm. Where else in RNA viruses, the capsid assembly will happen in cytoplasm except for retroviruses where this process will take place at plasma membrane (Martin, 2009). In the case of icosahedral virus, empty procapsids are first formed before the nucleic acids inserted in it. Even though the releasing process is different in enveloped and naked viruses, but the assembly step is generally similar in both enveloped and non-enveloped viruses (Michaela, *et al.,* 1995).

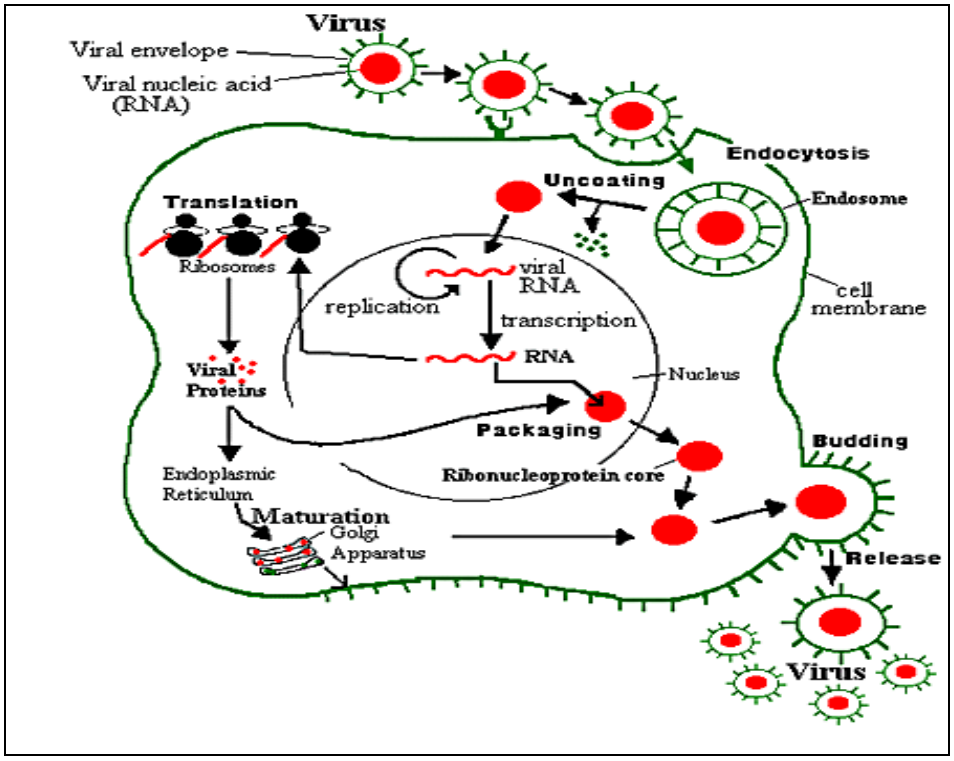


Figure 2.4: Common viral life cycle. The seven steps in viral life cycle include attachment, penetration, uncoating, replication, assembly, maturation and release. (Adapted from Kenneth, T. (2009). Textbook of Bacteriology)

2.3.3 Replication sites for DNA and RNA viruses

DNA or deoxyribonucleic acid is the major storage for genetic code that contains hereditary information for the functioning of all living organism on earth (Prescott, *et al.,* 1999). In is found in nucleus of each cell. Where else, RNA that stands for ribonucleic polymer acid performs a significant role in translating the genetic code from DNA to protein products. It is found in nucleus and as well as in cytoplasm (Casjens and King, 1975). The main difference between DNA and RNA is that DNA is a double stranded molecule with long nucleotide chain while RNA is a single stranded molecule with shorter nucleotide chain. In comparison with replication site, usually DNA viruses' replication takes place in the nucleus but poxviruses are exceptional cases since their genomes are replicated in cytoplasm (Kathleen, 1999). Following the procedure, the early mRNA will be transcribed from DNA by host enzymes but in poxvirus the early mRNA will be synthesized by a viral polymerase. Poxvirus is one of the special cases in DNA viruses (Casjens and King, 1975; William and David, 2002).

Whereas, the RNA viruses replication occurs in cytoplasm in which the RNA viruses are more diverse in their reproductive strategies than the DNA viruses. In fact, RNA viruses can be classified more in details based on their sense. The RNA viruses can be either positive sense (+ve RNA) or negative sense (-ve RNA). An example of +ve RNA viruses are picornaviruses while example of –ve RNA viruses are paramyxoviruses and orthomyxoviruses (Nayak, 1996 and William and David, 2002). Generally, in RNA viruses the nucleic acid replication will take place at cytoplasm but orthomyxoviruses that causes influenza is an exceptional case where the replication will happen at nucleus (Nayak, 1996).

2.4 Virus classification

The fact is that, the classification of viruses is less satisfactory compare to bacteria or eukaryotic classification. It is due to lack of their origin and evolutionary history (Ackermann, 1987 and Edward, 2006). Generally, viruses are classified based on their host preference which are animal, plant and bacteria. Besides host preference, viruses also can be classified upon their morphology. At the genome level, viruses can be group under DNA viruses and RNA viruses (Raven, *et al.,* 2005).

2.4.1 Baltimore classification scheme

The credit for developing the Baltimore classification scheme accredited to the Nobel Prize winning Virologist, David Baltimore. This classification is based on the viruses' genome type and their mechanism of transmitting their genetic information (Baltimore, 1971). In fact all viruses have the necessity to generate mRNAs from their genome in order to produce proteins and replicate themselves. To achieve this motive different mechanisms are used by viruses from each family according to their genetic code.

All together there are seven groups under this Baltimore scheme and the main focus is on the mechanism of the mRNA productions (Figure 2.5). The viruses are grouped into a method devised by David Baltimore. The groups are as following; group I (dsDNA viruses), group II (ssDNA viruses), group III (dsRNA viruses), group IV (+ssRNA viruses), group V (-ssRNA viruses), group VI (ssRNA-RT viruses) and group VII (dsDNA-RT viruses) (Baltimore, 1971 and Ackermann, 2003).

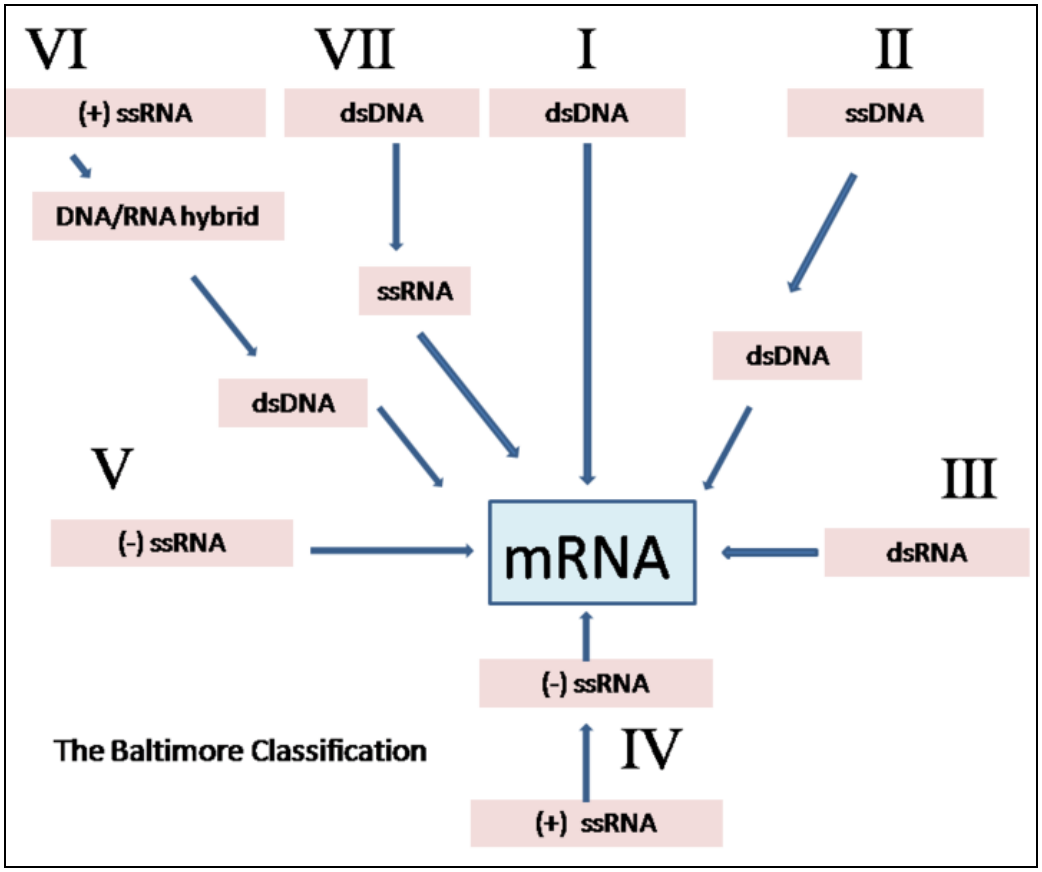


Figure 2.5: The Baltimore classification of viruses. (David, 1971).

In comparison with International Committee on Taxonomy of Viruses classification, viruses are basically classified based on Linnaean hierarchical system. The general taxonomy structure in ICTV starts with order (virales), family (viridae), subfamily (virinae), genus (virus) and species (virus). The similarity between the Baltimore and ICTV classification is that, the viruses are grouped based on the type of nucleic acids forming in their genome. Even though there are some differences between these two systems but both the classification systems have their major role to classify viruses accordingly (ICTV, 7TH Report and Ackermann, 2003).

2.5 Introduction to bacteriophage

All the world's a phage – W.Shakespeare

The word "phagein" in Greek stands for bacteriophage which means to eat. Bacteriophages are viruses that act as bioagent to target and destroy disease causing bacteria. The credit of discovering viruses specific to bacteria goes to Sir Frederick Twort and Felix d'Herelle in the 20th century (Shigenobu, *et al.,* 2005 and Edward, 2006). In 20th century (1915-1917), Sir Frederic Twort and Sir Felix d'Herelle were first to introduce the bacteriophage. It is known to be a virus that able to infect and lyze the bacteria. Sir d'Herelle independently noted that when a virus suspension was spread on a layer of bacteria lawn on agar, clear circular zones formed that contains viruses (Kathleen, 1999). A count of these clear zones allowed him to estimate the number of viruses present and the process was named as plaque assay.

These findings had given chance scientist to study the structure, genetics and replication of virus. All microorganisms under group of viruses contain same genetic information including phages. The genetic material of phage can be ssRNA, dsRNA, ssDNA or dsDNA (Marisa, 2003). Among the discovered phages, 95% are reported to have double stranded DNA as genome and possibly make up the majority of phages on the planet (Ackermann, 2003 and Pedulla, *et al.,* 2003). The type of nucleic acids and the site of nucleic acids replication vary depending upon the phage. The most complex phages may code for over 100 gene products while the simplest phages only have nucleic acid to code for 3-5 average size gene products (Xiao, *et al.,* 2009)

In structure basis, mostly identified phages are tailed and the tail is a hollow tube through which the nucleic acid passes during infection. Apart from the tail, all phages consists head structures which vary in size as well as shape. Mostly are icosahedral and some are filamentous (William and David, 2002). The differences between icosahedral and filamentous phages showed in Figure 2.6. The head or also known as capsid made of number of copies of one or more different proteins. Nucleic acid resists inside the head of phages. More over, the function of the capsid is to protect the nucleic acids of phages (Shigenobu, *et al.,* 2005).

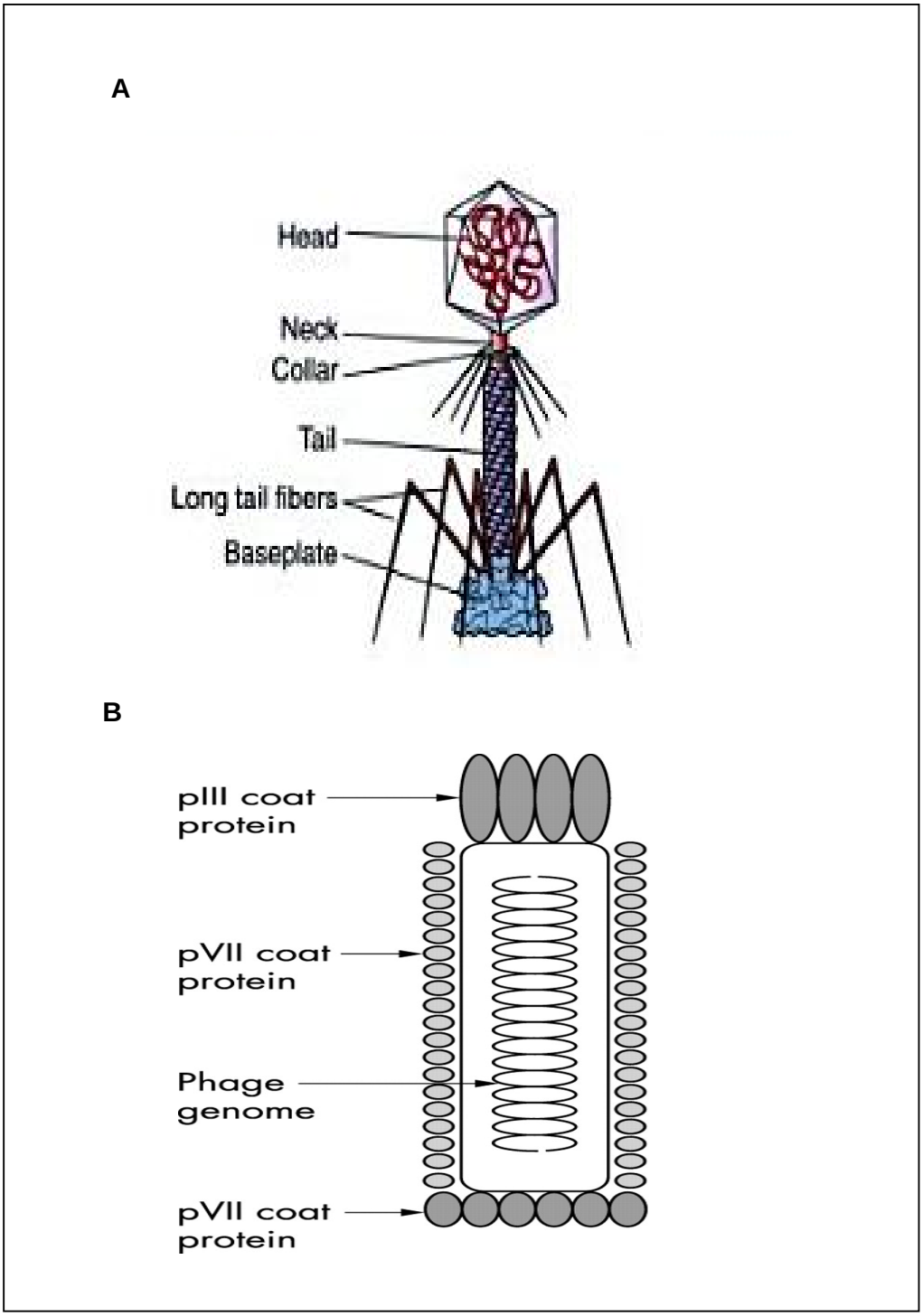


Figure 2.6 : Basic bacteriophage structure. (A) Icosahedral bacteriophage and (B) filamentous bacteriophage. (Adapted from Tropp, B.E. 2008 and Smith, et al., 2004)

Although phages are categorized under group of virus but there are significant different between phages to animal and plant viruses. The difference is based on the approach the viral nucleic acids enter their host (Ackermann and Dubow, 1987). Fundamentally in life cycle of a virus there are seven major steps but in the case of phage there are only six stages (Summer, 2001). The phages do not have an uncoating step since only their nucleic acid will penetrate into the host cellular environment not the whole capsid as in other viruses. Another diverse is that phages have two types of life cycle, it can be either lytic or lyzogenic (temperate) life cycle. Based on this criterion, phages can be further classified as virulent phages or temperate phages (Summer, 2003 and Shigenobu, *et al.,* 2005).

2.6 Bacteriophages classification

Bacteriophages were discovered twice at the beginning of the 20th century (Shigenobu, *et al.,* 2005 and Andrew, 2006). According to d'Herelle, there was one bacteriophage species with many races (d'Herelle, 1949 and Ackermann, 2003). In the 1967, the first phage survey indicated that at that year there were 111 negatively stained phages. Among the 111 phages 99 were tailed, 9 cubic and 3 filamentous and this identification was done with aid of an electron microscope (Eisenstark, 1967 and Ackermann, 2001). At presently, ICTV had classified phages in to 1 order (*Caudovirales*) with 13 families and 30 genera under this virale. The established 3 families are *Myoviridae,Siphoviridae* and *Podoviridae*. The remaining 10 families of phages are still not assigned to an order (ICTV latest update 26, March 2011 and Ackermann, 2003).

It is estimated there are ~6000 phages and up to end of 20th century 5300 bacterial viruses had been examined under electron microscope (Ackermann, 2001 and Shigenobu, *et al.,* 2005). In comparison with other viruses, bacteriophages constitute the largest viral group in nature. About 96% of reported phages are tailed phage, so there are 4950 tailed phages in numbers (Ackermann, 2001). The large numbers of phages are categorized under the 3 main families of *Caudovirale.* The remaining 190 phages (3.6%) are either filamentous, cubic or pleomorphic in morphological feature. These phages are classified into the 10 small families which differ by the most basic properties. The 10 familes are *Microviridae, Corticoviridae, Tectiviridae, Leviviridae, Cystoviridae, Iniviridae, Lipothrixviridae, Rudiviridae, Plasmaviridae and Fuselloviridae* (Table 2.1) *(*Ackermann, 2001 and Pedulla, *et al.,* 2003).

Table 2.1: Bacteriophage taxonomy based on 2010 International Committee on Taxonomy of viruses (ICTV)

Order	**Family**	**Shape**
Caudovirales	*Myoviridae* *Siphoviridae* *Podoviridae*	Tailed
Not assigned to an order	*Microviridae* *Corticoviridae* *Tectiviridae* *Leviviridae* *Cystoviridae* *Iniviridae* *Lipothrixvirid* *Rudiviridae* *Plasmaviridae* *Fuselloviridae.*	Filamentous / Polyhedral / Pleomorphic

2.7 Bacteriophages life cycle

In general, phages can be classified based on their multiplication cycle aide from their structure and morphology. There are two different types of phage life cycles. It can be either lytic cycle or lysogenic cycle. Figure 2.7 illustrate on life cycles of bacteriophage. Phages that undergo lytic cycle known to be virulent phage while phage that undergoes with lysogenic cycle called as temperate phages (Shigenobu, *et al.,* 2005).

Lytic cycle is a process whereby once the viral DNA enters into the host cells, it transcribes itself into the host cell's mRNAs. Basically, the host cell's DNA is destroyed and the virus' take over the host metabolic activities (Summer, 2001). Once the viruses been the controller in the host cell they will start to replicate and produces the new virion particles. Once the capacity of the host cell is full the virus will excrete enzymes to break the cell wall. This will cause the cells to burst and the newly formed virions will release (Young, *et al.,* 2000). This process is called as lysing and therefore the cycle is known to be lytic life cycle

However, temperate phages that undergo lysogenic cycle can either multiply via the lytic cycle or enter a quiescent cycle state in the cell (Ackermann and Dubow, 1987). If the temperate phages undergo lytic cycle it will lead to propagation and lyses but this does not occurs in all cases. In most of the time, the temperate phages that undergoes lysogenic cycles, either integrated into the bacterial chromosome or remains separate as a 'plasmid'. If the phage genome integrated into the bacterial

genome, eventually the phage genome multiplies cooperatively with the host bacteria without destroying it (Edward, 2006 and Martin, 2009).