The bacteriophage mu lysis system–A new mechanism of host lysis?

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Abstract: Bacteriophages are viruses that infect bacteria and can choose any one of the two alternative pathways for infection, i.e., lysis or lysogeny. Phage lysis is one of the conventional biological processes required to spread infection from one bacterium to another. Our analysis suggests that in the paradigm bacteriophage Mu, six proteins might be involved in host cell lysis. Mu has a broad host range, and Mu-like phages were found in both Gram-negative and Gram-positive bacteria. An analysis of the genomes of Mu and Mu-like phages could be useful in elucidating the lysis mechanism in this group of phages. A detailed review of the various mechanisms of phage lysis and different proteins associated with the process will help researchers understand the phage biology and their life cycle in different bacteria. The recent increase in the number of multidrug-resistant (MDR) strains of bacteria and the usual long-term nature of new drug development has encouraged scientists to look for alternative strategies like phage therapy and the discovery of new lysis mechanisms. Understanding the lysis mechanism in the Mu-like phages could be exploited to develop alternative therapeutics to kill drug-resistant pathogenic bacteria. In this review article, we have analyzed the phage Mu-mediated host lysis system, which is unknown till now, and our analysis indicates a possibility of the existence of a new lysis mechanism operating in Mu.

Introduction

The lysis of bacterial hosts by bacteriophages is a highly regulated process, controlled by several proteins, which act sequentially to disrupt the multi-layers of the bacterial membrane in a highly coordinated manner (Fig. 1). It has been proposed that two phage proteins play a crucial responsibility in phage lysis, and they are the endolysin and holin (Young, 1992). Endolysins induce the lysis pathway by breaking the peptidoglycan (PG) layer (Cahill and Young, 2019), but they need another group of proteins for access to the PG layer, and this class of proteins is known as holins. Holins are membrane-bound proteins, which form large pores in the inner membrane and allow the passage of endolysin to the PG layer (Young, 1992). Another phage-derived protein spanin is found to disrupt the outer membrane of Gram-negative bacteria (Kongari et al., 2018). In recent years, phage-derived endolysins and other PG hydrolases that disrupt the bacterial cell wall were found highly efficient in the treatment of bacterial infections, especially for Gram-positive bacteria (Fischetti, 2018; Kashani et al., 2018). The use of phage lytic proteins has shown promising results in clearing Methicillin-resistant Staphylococcus aureus (MRSA) infection in animal models (Gutiérrez et al., 2018). The unique ability of endolysins to quickly kill bacteria has enabled researchers to engineer them to be utilized as antibacterial or biocontrol agents in food preservation, bioprocess, fermentation, biotechnology, medicine, and others (Oliveira et al., 2012). The applications of endolysins were perceived to be only useful in Gram-positive bacteria as these bacteria lack the outer membrane of Gram-negative bacteria and, therefore, endolysins cannot directly access the PG layer in Gram-negative bacteria from outside. However, phage-derived novel endolysins were recently discovered that kill Gram-negative bacteria (Larpin et al., 2018; Lood et al., 2015).

The bacteriophage Mu is known to infect and kill a wide range of bacterial hosts (Paolozzi and Ghelardini, 2006). Moreover, several Mu-like phages were discovered in both...
Gram-negative and Gram-positive bacteria (Braid et al., 2004; Toussaint, 2013). However, the host-lysis mechanism of phage Mu is still unknown despite knowing the phage for more than 50 years by the scientific community (Taylor, 1963; Harshey, 2012; Saha et al., 2013). Our bioinformatics analysis of Mu genes revealed that at least seven gene products spanning early (kil), middle (19 and 20), and late regions (lys, 23/23a, and 25) of the Mu genome might be involved in host lysis. Our analysis also indicates a possibility that a new lysis mechanism may be operating in phage Mu because, in addition to putative holin, endolysin, and spanin proteins, other putative lysis-related proteins are also found in the genome (Faelen and Toussaint, 1973; Mathee and Howe, 1993; Morgan et al., 2002; Summer et al., 2007; Pastagia et al., 2013). Understanding how lysis proteins operate in Mu/Mu-like phages may provide us useful information that can be utilized to genetically engineer new lysis proteins or broad-range lytic phages for controlling multidrug-resistant bacterial infections.

The Bacteriophage Lysis System

Endolysins induce the lysis pathway by breaking the peptidoglycan layer (Cahill and Young, 2019). But these proteins generally do not possess any signal sequence and are unable to pass the cytoplasmic membrane by themselves (Cahill and Young, 2019). They need another group of proteins for access to the peptidoglycan layer, and this class of proteins is known as holins. Holins are membrane-bound proteins, which form large pores or holes in the cytoplasmic or inner membrane and allow passage for endolysin to the peptidoglycan layer (Young, 1992). For more than two decades, researchers perceived this mechanism as the sole pathway for phage lysis (Figs. 1A, 1B, and 1E). However, the holin-endolysin theory lost its universality upon the discovery of a new category of proteins, pinholin/SAR (signal-arrest-release) endolysin, that emerged as alternative candidates for phage lysis (Figs. 1C, 1D, and 1E). Despite having similarities in the naming pattern, the molecular mechanisms of action of these two sets of enzymes are radically different (Young, 2013). SAR endolysin does not need holins for their transport to the peptidoglycan layer, and instead, they are reported to carry the N-terminal SAR sequence, which enables them to transport through the membrane by bacterial sec system (São-José et al., 2000). So, SAR endolysins could be transported to the periplasm in a holin-independent manner. However, these membrane-bound SAR endolysins are in inactive form unless they are released from the membrane and refolds in a proper active conformation (Sun et al., 2009; Xu et al., 2005). Their release from the membrane is correlated with their activation, and this is done by rapid membrane
depolarization (Cahill and Young, 2019). The rapid membrane depolarization is mainly dependent on another group of proteins, i.e., pinholins. Instead of transporting endolysin, pinholins form small pores in the membrane, which help in rapid depolarization of the membrane and consequently activation of the SAR endolysins (Young, 2013). Another class of proteins named antiholins inactive the holin assembly until the phage is ready to enter the lytic cycle (Wang et al., 2000; Park et al., 2006).

In addition to the holin-endolysin theory, some other lytic proteins play a pivotal role in the lysis of Gram-negative bacteria, named spanins (Kongari et al., 2018). They are associated with the disruption of the third layer, i.e., the outer membrane of Gram-negative bacteria. There are two classes of spanin proteins (one-component and two-component systems) reported so far. The two-component spanin complex consists of two proteins, i.e., α-spanin and i-spanin. O-spanin is an outer membrane lipoprotein, whereas i-spanin is an essential cytoplasmic membrane protein, and these two proteins are connected by a periplasmic domain (Young, 2013). One-component spanin system is known as u-spanin, which consists of a C-terminal transmembrane domain as well as an N-terminal outer membrane signal. The primary function of the spanins is to disrupt the outer membrane of Gram-negative bacteria and to fuse the inner and outer membranes for facilitating host cell lysis.

Unlike the phages of Gram-negative bacteria, lytic phages of Gram-positive bacteria do not need spanin proteins to promote cell lysis (Fig. 1F). However, it has been observed that overexpression of lytic genes (holin and endolysin) from Lactobacillus fermentum temperate bacteriophage phiPYB5 exhibited a broad range of lytic spectrum and can induce lysis in both Gram-positive and Gram-negative bacteria (Wang et al., 2008). These proteins could potentially be exploited as a therapeutic method against a broad range of pathogenic bacteria. Some endolysins secreted by phages that infect Gram-positive bacteria (e.g., Lactobacillus plantarum phage φg1e, Oenococcus oeni phage fOg44, and Bacillus cereus phage TP21-L, etc.) contain signal sequences (intrinsic) that permit them to go through the bacterial inner membrane (Kakikawa et al., 2002; Sá-José et al., 2000). Lysins or endolysins released by phages of Gram-positive bacteria usually have modular conformation defined by N-terminal catalytic domains (one or more with different specificities) and C-terminal cell wall-binding domains (Braid et al., 2004; Fenton et al., 2010; Fischetti, 2005; Pastagia et al., 2013).

Also, specific enzymes of mycobacteriophage have been identified that are known to target the complex cell wall of Mycobacterium. The lytic enzymes of mycobacteriophage mainly comprise lipolytic enzymes that target the mycolic acid-containing outer membrane of the bacteria and peptidoglycan hydrolases that are responsible for breaking the mycobacterial peptidoglycan layer (Fig. 1G) (Catalão and Pimentel, 2018). The mycobacteriophage Ms6 that infects Mycobacterium smegmatis has several proteins that control host lysis-Gp1 assists the transport of the endolysin (LysA) utilizing the host sec system, and the lipolytic LysB protein that disrupts the OM of mycobacteria (Catalão and Pimentel, 2018).

Unlike dsDNA lytic phages, ssRNA and ssDNA lytic phages do not possess multiple lysis proteins. Instead, the host lysis process was facilitated by a ‘single-gene lysis’ (Sgl) protein that mainly inhibits bacterial cell wall synthesis (Chamakura and Young, 2019). The sgl genes share little to no sequence similarity to each other and are sometimes found more than one per phage genome (Chamakura et al., 2020; Chamakura and Young, 2020).

**Mu Lysis System**

To identifying the lysis genes that are present in bacteriophage Mu, the genome sequence of Mu has been downloaded from the Nucleotide database (Benson et al., 2013) (GenBank: AF083977.1) and was analyzed for the genes that may affect the host lysis process (Braid et al., 2004; NCBI Resource Coordinators, 2018; Morgan et al., 2002). All Mu protein sequences were searched for homologs by the BLAST program against the UniProtKB database using the UniProt server (Altschul et al., 1990; Pandur et al., 2017; Uniprot Consortium, 2019). The presence of any conserved domain in protein sequence was searched by the NCBI CDD (conserved domain database) server (Marchler-Bauer et al., 2017). The presence of transmembrane domains and signal sequences in the Mu proteins were searched using TMPred and Protter servers (Hofmann and Stoffel, 1993; Omasits et al., 2014).

The bacteriophage Mu dsDNA genome spans 36,717 base pairs comprising at least 56 genes that are divided into three regions based on their expression timings in life-cycle: early, middle, and late (Morgan et al., 2002) (Fig. 2). The genes present in the early region are involved in controlling lytic-lysogenic choice, transposition, and replication, middle region genes are required for transcriptional regulation, and late region genes are responsible for encoding structural proteins of the phage (Choi et al., 2014; Harshay, 1988; Morgan et al., 2002). The region between muB and muC genes consists of 16 other genes (kil, 6, 7, 8, 9, gam, 11, 12, 13, 14, 15, gemA, mor, 18, 19, and 20) (Fig. 2), spanning...
about 5.5 kb region, whose functions are not essential for the phage Mu and was named as the “semi-essential” (SE) region (Morgan et al., 2002; Paolozzi, 1987). The host-lysis mechanism by bacteriophage Mu is still a mystery, and we predicted that at least seven gene products spanning early (kil), middle (19 and 20), and late (lys, 23/23a, and 25) regions of Mu’s genome may be involved in host-lysis (Tab. 1, Fig. 2).

Interestingly, three (kil, 19, and 20) out of these seven predicted lysis genes fall within the SE region of the Mu genome.

In bacteriophage Mu, the expression of the Mu kil gene causes the death of host cells in the absence of phage replication (van de Putte et al., 1977; Westmaas et al., 1976). Expression of the kil gene induces striking morphological changes in the host cell structure, which becomes enlarged and mostly spherical, resulted in cell death (Waggoner et al., 1989). Although there is no clear idea of how the expression of the kil gene leads to cell death, some data suggested that the lethal effect of kil comes from its interaction with the cell wall, which resulted in abnormal cell wall synthesis (Goosen and van de Putte, 1984; Paolozzi, 1987). The deletion of a region of the Mu genome stretching from 4.4 kb to 7.2 kb (from kil to gam gene) resulted in tiny plaques after an abnormally long latent period of 4–5 h (Pastagia et al., 2013) compared to the normal period of 1 h (Goosen et al., 1982). This extended latent period can be substantially reduced by expressing SE genes from a recombinant plasmid, and even only expression of the kil gene product alone is sufficient to restore the latent period almost to normal (Goosen et al., 1982; Waggoner et al., 1989).

Bacteriophage λ and Rac prophage each hold a gene, also known as kil (Conter et al., 1996; Haeusser et al., 2014). However, these kil genes of λ or Rac phages do not have any sequence or functional similarity with the Mu kil gene (Waggoner et al., 1989; Haeusser et al., 2014). The expression of Rac kil stops FtsZ ring formation, which inhibits cell division that results in filamentation of the cell (Conter et al., 1996). The product of bacteriophage λ kil inhibits host cell division via the ZipA-dependent inhibition of FtsZ joining (Haeusser et al., 2014).

Mor (middle operon regulator) protein that acts as the activator for the Pm promoter transcribes 4 genes—18, 19, 20, and C (Fig. 2). The C protein activates the Plys promoter that transcribes 10 genes, from lys to G genes (Howe, 1998; Margolin and Howe, 1990; Margolin et al., 1989; Swapna et al., 2015; Zha et al., 1994). The lys gene product of Mu was found to be essential for efficient host cell lysis, although the mechanism is not known (Faelen and Toussaint, 1973). The lys gene appears to encode the Mu endolysin protein due to its high similarity with endolysins found in other phages (Tab. 1). The NCBI CDD search of Mu lys gene product identified it as a member of the Lyz-like superfamily that includes proteins with lysozyme-like domains, for example, lysozyme, endolysin, pesticin, etc. (Marchler-Bauer et al., 2017). We also found that the lys gene sequence has an N-terminal signal-arrest-release (SAR) sequence, which is a characteristic of SAR-endolysins (Xu et al., 2004). The UniProt server also annotated the Mu lys gene product as a putative SAR-endolysin (Tab. 1).

Interestingly, three out of four gene products (18, 19, and 20) of Pm promoter induced transcript were found to have some role in host cell lysis, as deletion of these genes resulted in 6–22 min delay in host cell lysis, but that did not affect plating efficiency and burst sizes. Therefore these three genes were identified as ‘non-essential’ genes for Mu (Mathee and Howe, 1993). The 18 gene product does not appear to have any transmembrane domain or signal-sequence, and therefore, unlikely to have any effect on the host lysis system, as no role within the host lysis system has yet been established. The 19 gene product was predicted to be similar to E. coli FhuB protein that scavenges iron and increases pathogenicity of the host, thereby resulted in a fitness advantage (Crosa, 1989). The 19 gene product has four transmembrane helices and a highly charged hydrophilic C-terminal end as predicted by our bioinformatic analysis and appears to be a new type of holin as most of the holins known were usually consist of up to 3 transmembrane helices (Park et al., 2006; To and Young, 2014; White et al., 2011). However, putative holins with four transmembrane helices have been identified in Mycobacterium phage Acadian, TM4, and in others too (Reddy and Saier, 2013). Holins usually have a simple membrane topology with membrane-spanning helices and a highly charged C-terminal end (Young and Bläsi, 1995; Reddy and Saier, 2013). Based on the membrane topology holins were mainly segregated into three classes—I, II, and III (Park et al., 2006; To and Young, 2014). Class I holin has three transmembrane domains (e.g., phage λ S holin, phage P2 Y holin, etc.), class II has two transmembrane domains (e.g., phage 21 S21 holin, etc.), and class III has a single transmembrane domain (e.g., phage T4 T holin, etc.) (Park et al., 2006; To and Young, 2014; White et al., 2011). Interestingly some class I and class II holin genes encode two proteins—one acts like a holin and the other as antiholin (White et al., 2011). Phage λ S holin gene (class I holin) encodes two proteins, S107 and S015, where S107 acts as an antiholin by inhibiting S105 mediated membrane disruption (Park et al., 2006). The additional positive charge in the N-terminal of S107 restricts the movement of its first transmembrane domain through the membrane, and in that conformation, it can inhibit the S105 holin (Park et al., 2006). Similarly, in the phage 21 S21 holin gene (class II holin) encodes two proteins, S2171, and S2168, where S2171 and S2168 are antiholin and holin, respectively (Park et al., 2006). The 20 gene product is a putative membrane protein with a single transmembrane helix and might also be involved in host lysis as deletion of the gene resulted in delayed host lysis (Mathee and Howe, 1993) (Tab. 1). The presence of transmembrane helix and N-terminal charge residues are characteristic features of an antiholin protein, which are also the characteristic features of gene 20 product (Young and Bläsi, 1995).

The 23 gene product (23/23a overlapping genes) was predicted to be the equivalent of phage spanin (i-spanin and o-spanin) proteins (Summer et al., 2007). Our bioinformatic analysis also finds that the product of gene 25 is a transmembrane protein and may have some role in the host lysis. The TMpred software finds the presence of one transmembrane domain in the gene 25 product (Tab. 1). Also, the bioinformatic analysis revealed both 23/23a and 25 gene products have N-terminal signal sequences (Tab. 1). In
# TABLE 1

The putative lysis genes of bacteriophage Mu

| No. | Gene name | Amino Acids (aa) | Uniprot ID | TM Helix^a^-Protter & TMpred | Conserved domain | UniProtKB | Function (Known/Putative) |
|-----|-----------|------------------|------------|----------------------------|------------------|----------|--------------------------|
| 1   | kil       | 74               | P03046     | None                       | None             | kil from *E. coli* phage D108 | C9DGL3 1.9 × e^-58 100.0 | Involved in host cell killing (*Goosen and van de Putte, 1984; Waggoner et al., 1984; Waggoner et al., 1989*) |
| 2   | gene_19   | 120              | Q38646     | Present (four)              | None             | gene_19 from *E. coli* phage D108 | A0A0C4UQY4 1.9 × e^-37 98.6 | Putative holin (*Reddy and Saier, 2013*) |
| 3   | gene_20   | 39               | Q38623     | Present (one)               | None             | gene_20 from *E. coli* phage D108 | A0A0C4UQY7 4.6 × e^-28 97.4 | Putative membrane protein (*Mathee and Howe, 1999*) |
| 4   | lys       | 171              | Q9T1X2     | Present (Two; N-terminal signal peptide present) | Lys-like super family | lys from *E. coli* phage D108 | C9DGM9 2.3 × e^-120 99.4 | Putative SAR-endolysin (*Faelen and Toussaint, 1973; Xu et al., 2004; Marchler-Bauer et al., 2017*) |
| 5a  | gene_23   | 128              | Q9T1X1     | Present (one; N-terminal signal peptide present) | None | gene_23 from *E. coli* phage D108 | A0A0C4UQY4 4.4 × e^-118 97.7 | Putative Rz (1-spanin) (*Summer et al., 2007*) |
| 5b  | gene_23a  | 112              | -          | Present (two; N-terminal signal peptide present) | None | Rz1 from *Shigella* phage SIV | Q8SBX8 1.0 × e^-10 47.2 | Putative Rz1 (o-spanin) (*Summer et al., 2007*) |
| 6   | gene_25   | 99               | Q9T1W9     | Present (one; N-terminal signal peptide present) | DUF2730 super family | gene_25 from *E. coli* phage D108 | A0A0C4UQY7 2.1 × e^-37 95.9 | Putative membrane protein (*Ramanculov and Young, 2001*) |

Note: Amount of putative transmembrane helix/helices present is indicated within bracket; Homologous genes present in other phages (>45% identity); Percentage identity with corresponding Mu gene.

Note: aNumber of putative transmembrane helix/helices present is indicated within bracket; bHomologous genes present in other phages (>45% identity); cPercentage identity with corresponding Mu gene.
T4 phage, rI gene encodes an antiholin that possesses an N-terminal signal sequence similar to gene 25 product in Mu (Ramanculov and Young, 2001). Therefore, the gene 25 product may either function as an antiholin or it may have a novel function involving host lysis (Young and Bläsi, 1995). It is not clear whether phage Mu has a holin-endolysin or SAR-endolysin-pinholin system. The lys gene product has all the features to be a SAR-endolysin protein, and the 19 gene product is predicted to be a holin, but there is no known SAR-endolysin-holin system in bacteria. Therefore, the gene 19 product may also function as a pinholin to complete the SAR-endolysin-pinholin pairing (Fig. 1C). However, in phage P1, the Lys protein which encodes a SAR-endolysin found to be sufficient to lyse the host cell even though the phage expresses a holin (LydA) and an antiholin (LydB) (Young, 2014). Moreover, in phage P1, the lys gene is not clustered with holin lydA or antiholin lydB genes (Young, 2014), similar to phage Mu lys gene (late region gene), which is also not clustered with the putative holin 19 gene (middle region gene) (Morgan et al., 2002), indicating that the Mu Lys protein may also function in a holin-independent manner similar to P1 Lys. At this point, it is not clear whether the gene 20 or gene 25 product functions as an antiholin or they might have a novel lysis function. Further experiments are necessary to ascertain their functions. Unlike any other phage, the Mu early (kil) and middle (19 and 20) region genes, although ‘semi-essential’, have some indirect effects on the phage lytic cycle as deletion of these gene delays host lysis (Mathee and Howe, 1993; Pastagia et al., 2013). Together, the Mu lysis system is appeared to be less similar to other lambdoid and T4 systems (Young, 1992), and the data suggests that there is a possibility of a new lysis mechanism operating in phage Mu which needs to be uncovered for a better understanding of the underlying mechanism.

**Mu-like Phages**

Several Mu-like transposable phages were discovered not only in Gram-negative bacteria, e.g., *Escherichia coli* phages D108 and Sp18 (Hayashi et al., 2001; Hull et al., 1978), *Pseudomonas aeruginosa* phages D3112, B3, PaMx73 and H70 (Braid et al., 2004; Cazares et al., 2014; Roncero et al., 1990), *Haemophilus influenzae* phage FluMu (Fleischmann et al., 1995), *Neisseria meningitidis* phages Pnm1, Pnm2 and MuMenB (Klee et al., 2000; Massignani et al., 2001), *Salmonella typhi* phage SalMu (Parkhill et al., 2001), *Photobacterium luminescens* phage PhotoMu (Duchaud et al., 2003), *Chromobacterium violaceum* phage ChromoMu (Brazilian National Genome Project Consortium, 2003), *Burkholderia cenocepacia* phage BcepMu (Summer et al., 2004), *Burkholderia cepacia* phage KS10 (Goudie et al., 2008), *Rhodobacter capsulatus* phage RcpMu (Fogg et al., 2011), *Haemophilus parasuis* phage SuMu (Zehr et al., 2012), *Shigella flexneri* phage SFMu (Jakheta and Verma, 2015), *Mannheimia haemolytica* phage 3927AP2 (Niu et al., 2015), etc., but also in Gram-positive bacteria, e.g., *Deinococcus radiodurans* phage RadMu (White et al., 1999), *Syntrophobatus glyclicus* phage SglyMu-1 (Toussaint, 2013), *Bacillus alcalophilus* phage BalMu (Yang et al., 2015), etc. (Tab. 2). These Mu-like phages all carry proteins that control lysogenic or lytic lifestyle, ‘semi-essential’ genes with unknown functions, the transposition activator B as well as transposase A genes, head and tail genes, lysis genes, etc. Overall, till now, more than 50 Mu-like

**TABLE 2**

Partial list of Mu-like phages found in Gram-negative (-) and Gram-positive (+) bacteria

| No. | Phage name | Phage family | GenBank accession No. | Phage genome size | Reference |
|-----|------------|--------------|-----------------------|-------------------|-----------|
| 1   | *Escherichia coli* phage D108 (-) | Myoviridae | NC_013594 | 37235 bp | Hull et al., 1978 |
| 2   | *Pseudomonas aeruginosa* phage D3112 (-) | Siphoviridae | NC_005178 | 37611 bp | Roncero et al., 1990 |
| 3   | *Pseudomonas aeruginosa* phage B3 (-) | Siphoviridae | NC_006548 | 38439 bp | Braid et al., 2004 |
| 4   | *Pseudomonas aeruginosa* phages PaMx73 (-), and H70 (-) | Siphoviridae | JQ067085 (PaMx73); NC_027384 (H70) | 36570 bp (PaMx73); 37359 bp (H70) | Cazares et al., 2014 |
| 5   | *Burkholderia cenocepacia* phage BcepMu (-) | Myoviridae | NC_005882 | 36748 bp | Summer et al., 2004 |
| 6   | *Burkholderia cenocepacia* phage KS10 (-) | Myoviridae | NC_011216 | 37635 bp | Goudie et al., 2008 |
| 7   | *Rhodobacter capsulatus* phage RcpMu (-) | Siphoviridae | NC_016165 | 39283 bp | Fogg et al., 2011 |
| 8   | *Haemophilus parasuis* phage SuMu (-) | Myoviridae | NC_019455 | 37151 bp | Zehr et al., 2012 |
| 9   | *Shigella flexneri* phage SFMu (-) | Myoviridae | NC_027382 | 37146 bp | Jakheta and Verma, 2015 |
| 10  | *Bacillus alcalophilus* phage BalMu (+) | Myoviridae | NC_030945 | 39873 bp | Yang et al., 2015 |
phages were discovered that reside or infect as prophages in both Gram-negative and Gram-positive bacteria, indicating that they are widespread mobile genetic elements in nature (Braid et al., 2004; Toussaint, 2013). Unfortunately, no work has been done on the lysis-system operating in these Mu-like phages infecting Gram-negative and Gram-positive bacteria. Therefore, analysis of these Mu-like phage genomes is essential and would help us to understand how the lysis machinery working in this unique group of bacteriophages.

All predicted lysis genes in phage Mu were found to be highly similar (>97%; Tab. 1) to Escherichia phage D108 homologs even though these two phages have dissimilar host ranges and were hetero-immune (Hull et al., 1978). The Shigella phage SfMu is also found to be very close to phage Mu as most of its proteins have >95% sequence similarities with phage Mu (Tab. 1) (Jakheta and Verma, 2015). In other Mu-like phages, FluMu (Haemophilus), D3112 (Pseudomonas), and PaMx73 (Pseudomonas), most of the genes were found to be conserved, including semi-essential and lysis genes (Cazares et al., 2014; Morgan et al., 2002). Similarly, several other Mu-like phages in Gram-positive and Gram-negative bacteria (e.g., Pseudomonas phage B3, Mannheimia phage 3927AP2, Syntrophobotulus phage SglyMu, Bacillus phage BalMu, etc.) were found to encode proteins, including lysis proteins, that are homologous to phage Mu proteins (Braid et al., 2004; Niu et al., 2015; Toussaint, 2013; Yang et al., 2015), although, in some Mu-like phages variations in the early and middle regions of the genomes were observed (Morgan et al., 2002; Niu et al., 2015). Further analysis of the early, middle and late regions of the Mu-like phages is required to understand how these unique phages evolved with time especially in the context of Gram-negative and Gram-positive bacteria.

**Phage Therapy by Lytic Phage, or Endolysin Protein**

Various lytic phages or phage-derived endolysin proteins were found to successfully reduce or clear the fatal/multidrug-resistant infections by several pathogenic bacteria, e.g., *Pseudomonas aeruginosa* (Fong et al., 2016), *Enterococcus faecalis* (Cheng et al., 2017), *Salmonella enteritidis* (Chen et al., 2018), *Pseudomonas aeruginosa* (Jeon and Yong, 2019), *Acinetobacter baumannii* (Wu et al., 2019), *Klebsiella pneumoniae* (Thiry et al., 2019), *Escherichia coli* (Naghizadeh et al., 2019), *Staphylococcus aureus* (Zhang et al., 2018), *Salmonella typhimurium* (Seo et al., 2018), *Bacillus cereus* (Peng and Yuan, 2018), *Enterobacter aerogenes* (Zhao et al., 2019), *Mycobacterium abscessus* (Dedrick et al., 2019), etc (Tab. 3). Phage-derived endolysins and other peptidoglycan hydrolases that disrupt the bacterial cell wall are found to be highly efficient in the treatment of bacterial infections, especially for Gram-positive bacteria (Fischetti, 2018; Khashani et al., 2018; Nelson et al., 2001). The use of phage lytic proteins has shown promising results in clearing Methicillin-resistant *Staphylococcus aureus* (MRSA) infection in animal models without any apparent side effects (Gutiérrez et al., 2018). The distinctive capability of endolysins to quickly kill bacteria has enabled researchers to engineer them to be utilized as antibacterial or biocontrol agents in food preservation, bioprocess and fermentation, biotechnology, medicine, and others (Oliveira et al., 2012). Although the activity of endolysin is restricted in Gram-negative bacteria due to the existence of an impermeable outer membrane, the development of genetically engineered endolysins is in full force that kills Gram-negative bacteria (Briers and Lavigne, 2015; Briers et al., 2007; Briers et al., 2011; Vázquez et al., 2018). Also, phage-derived novel endolysins were found recently that kill Gram-negative bacteria (Larpin et al., 2018; Lood et al., 2015). The endolysin proteins are being engineered so that they can penetrate the outer membrane of Gram-negative bacteria and reach the peptidoglycan layer. It has been shown that the addition of a highly positive-charged peptide at any of the end of an exogenous lysis/endolysin can destabilize the outer membrane of Gram-negative bacteria to get access to the peptidoglycan layer which results in the killing of the bacteria (Larpin et al., 2018; Lood et al., 2015). These modified proteins work optimally at low pH when the terminal peptides become highly protonated, which destabilizes the outer membrane by interfering with the stabilizing divalent cations that interact with the negatively charged phosphate groups in the outer membrane (Larpin et al., 2018). Moreover, it has been observed that in recent times a considerable amount of progress has been done, specifically about the mechanism of action and detailed comprehension of bacteriophage lysis proteins, which could be considered as an important lead towards the development of alternative therapeutics specially designed to combat new multi-drug resistant pathogenic bacteria. For example, recently, researchers have developed a new generation of lytic proteins by adding selected peptides to endolysins named Artilysin (Rodriguez-Rubio et al., 2016). Artilysins are engineered endolysins fused with permeabilizing peptides that help the fusion protein to penetrate the outer membrane of Gram-negative bacteria and degrade the peptidoglycan layer resulting in the death of the bacteria (Briers et al., 2014). Artilysins are extremely bactericidal and found to be especially useful in killing a wide range of multidrug-resistant bacteria (Briers et al., 2014; Rodriguez-Rubio et al., 2016). It has been noted that these novel lytic proteins have a broad range of activities for both Gram-positive and Gram-negative bacteria.

Interestingly, Mu is the most efficient transposable element known (Mizuuchi, 1983; Walker and Harshay, 2020) and can infect and lyse several hosts (Escherichia coli, Shigella sonnei, Citrobacter freundii, Erwinia, and Enterobacter) (Paolozzi and Ghelardini, 2006). Moreover, Mu-like phages were discovered in a wide range of Gram-negative and Gram-positive bacteria (Hull et al., 1978; Hayashi et al., 2001; Toussaint, 2013). The lysis-system operating in Mu/Mu-like phages is predicted to be unique, and the presence of several putative lysis-related genes (holin/pinholin, SAR-endolysin, spanin, etc.) bolsters the hypothesis (Tab. 1). Deciphering the lysis mechanism operating in this group of phages may help us to design highly efficient lysis proteins that would kill a broad range of pathogenic bacteria.
**Conclusion**

Most phages require one or more of some specialized classes of proteins (holin/pinhoilin, endolysin, and spanin) that are designated to disrupt different layers of the cell envelope of the bacterial host (Young, 2014; Howard-Varona et al., 2017). Whereas, in simple phages (ssDNA and ssRNA phages), another diverse class of enzymes (single-gene lysis or Sgl proteins) is involved in host cell lysis. Phage encoded Sgl proteins are unique and are usually unrelated to each other (Chamakura and Young, 2019). Moreover, it has been shown that the lysis process has more important aspects than just the “rupture” of the cell wall, and a detailed understanding of phage lytic functions would be helpful in the development of advanced alternative drugs to combat present and new bacterial pathogens.

The phage Mu can infect and kill several different bacterial hosts (Paolozzi and Ghelardini, 2006), and Mu-like phages can lyse both Gram-positive and Gram-negative hosts (Hull et al., 1978; Hayashi et al., 2001; Toussaint, 2013). The lytic machinery of bacteriophage Mu is unknown, and our analysis reveals the presence of several putative lysis genes in Mu. The presence of ‘semi-essential’ lysis proteins (kil, 19, and 20 gene products) may help Mu phage to disrupt the integrity of the host cell membranes and, therefore, may assist in the host lysis process by the Mu SAR-endolysin Lys. The presence of these non-essential lysis genes might be the key element for the Mu/Mu-like phages for killing a wide range of hosts, both Gram-negative and Gram-positive bacteria. Therefore, understanding how the lysis system operates in Mu/Mu-like bacteriophages is of utmost importance as these proteins (e.g., endolysin, etc.) can be directly utilized to kill a broad range of bacteria, particularly multidrug-resistant bacteria. The possibility of the existence of a new lysis mechanism in phage Mu would be enticing enough for the researchers to uncover the long-standing mystery in this paradigm phage Mu.

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**Ethics Approval:** The research has not involved any animal or human.

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**TABLE 3**

Partial list of lytic phages found to kill pathogenic bacteria

| No. | Phage(s) | Pathogenic bacterium | Phage family | GenBank accession No. | Phage genome size | Reference |
|-----|----------|----------------------|--------------|-----------------------|------------------|-----------|
| 1   | Pa 193, Pa 204, Pa 222, Pa 223 | *Pseudomonas aeruginosa* | Myoviridae (193, 204); Podoviridae (222, 223) | NA* | NA | Fong et al., 2017 |
| 2   | EF-P29 | *Enterococcus faecalis* | Siphoviridae | KY30907 | 58984 bp | Cheng et al., 2017 |
| 3   | vB_SenS_CSP01, vB_SenS_PHB06, vB_SenS_PHB07 | *Salmonella enteritidis* | Demerecviridae (CSP01, PHB06); Drexlerviridae (PHB07) | KY114934 (CSP01); MH102285 (PHB06); NC_047947 (PHB07) | 117842 bp (CSP01); 84406 bp (PHB06); 51818 bp (PHB07) | Chen et al., 2018 |
| 4   | Bφ-R656, Bφ-R1836 | *Pseudomonas aeruginosa* | Siphoviridae | NC_028657 (R656); KT968832 (R1836) | 60919 bp (R656); 37714 bp (R1836) | Jeon and Yong, 2019 |
| 5   | PD-6A3 | *Acinetobacter baumannii* | Autographiviridae | NC_028684 | 41563 bp | Wu et al., 2019 |
| 6   | vB_KpnP_KL106-ULIP47, vB_KpnP_KL106-ULIP54, vB_KpnP_K1-ULIP33 | *Klebsiella pneumoniae* | Autographiviridae | MK380015 (ULIP47); MK380016 (ULIP54); MK380014 (ULIP33) | 41397 bp (ULIP47); 41109 bp (ULIP54); 41222 bp (ULIP33) | Thiry et al., 2019 |
| 7   | vB_EcoS_TM1, vB_EcoS_TM2, vB_EcoS_TM3, vB_EcoS_TM4 | *Escherichia coli* | Siphoviridae | NA | approx. 45.8 kb (TM1), 41.7 kb (TM2), 27.1 kb (TM3), 31.4 kb (TM4) | Naghizadeh et al., 2019 |
| 8   | Sa83, Sa87 | *Staphylococcus aureus* | Herleviridae | MK417514 (Sa83); MK417515 (Sa87) | 146004 bp (Sa83); 149108 bp (Sa87) | Zhang et al., 2018 |
| 9   | vB_BceM-HSE3 | *Bacillus cereus* | Myoviridae | MF418016 | 124002 bp | Peng and Yuan, 2018 |
| 10  | vB_EaeM_φEap-3 | *Enterobacter aerogenes* | Myoviridae | NC_041980 | 175814 bp | Zhao et al., 2019 |

Note: * NA = not available.
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