Anti-Restriction: Phage Strategy to Evade Restriction-Modification Systems

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Abstract. Bacteria, like all living organisms, are vulnerable to viral infections—specifically by viruses known as bacteriophages, or simply phages. The constant evolutionary arms race between phages and their bacterial hosts has led to the development of various bacterial defense mechanisms. Among these is the Restriction-Modification (R-M) system, a sophisticated strategy that enables bacteria to recognize and degrade invading foreign DNA. This study focuses on how phages have evolved to evade R-M systems. It involves compiling a comprehensive database of restriction enzymes and applying machine learning techniques to predict anti-restriction proteins encoded by phages. Through this approach, the research aims to enhance our understanding of phage-bacteria interactions and improve the computational prediction of phage evasion strategies. Ultimately, this work supports broader applications in phage therapy by integrating bioinformatics and machine learning to uncover how phages overcome bacterial defenses.

Keywords: Phage, Bacteria, Restriction Modification System (R-M), Phage-Host Arms Race, Machine Learning

1 Introduction

Bacteriophages (phages) are the most abundant organisms on the planet, outnumbering bacteria [1]. Since the early developments in molecular biology, the interaction between bacteria and phages has been thoroughly investigated [2].

1.1 Infection Cycles

Phages are viruses that infect bacteria and can only replicate in bacterial cells [3]. These viruses are composed of a nucleocapsid and a complex tail structure, and upon infection, they replicate through either a lytic cycle or a lysogenic cycle [4] [5], as described in Figure 1.

In the lytic cycle, also known as virulent infection, the infecting phage takes control of the host's cellular machinery to replicate its own genetic material and synthesize new viral particles. This process leads to the lysis of the host cell, releasing the new phages into the bacterial environment. In contrast, the lysogenic cycle, sometimes referred to as temperate or non-virulent infection,

allows the phage to inject its DNA into the host cell, integrating itself into the bacterial genome, forming a prophage. In this dormant state, viral DNA is passively replicated along with the bacterial genome as host cells divide, without causing damage to the host [6].

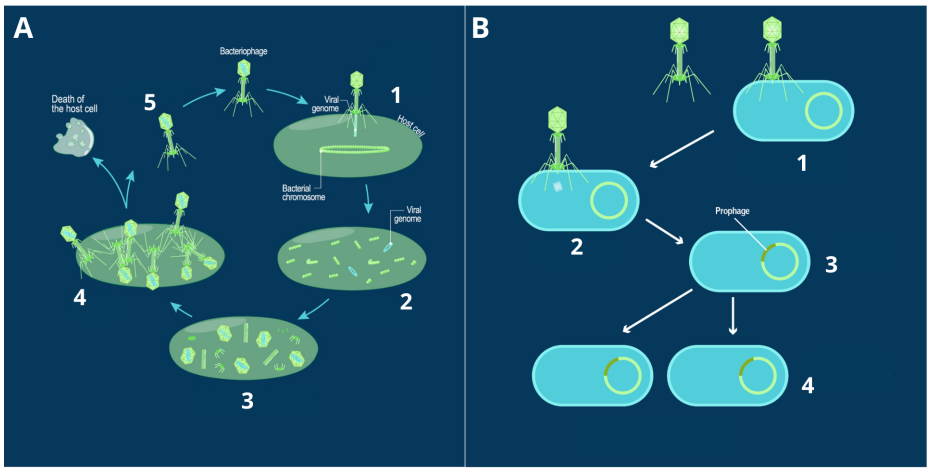

Fig. 1. Depiction of the Bacteriophage Life Cycles **A**- Lytic Cycle: (1) Phage attaches to a bacterial cell and injects its DNA; (2) Viral DNA takes over, breaking down the host's genome; (3) New viral components are synthesized; (4) Viruses are assembled and released as the host cell bursts (lysis); (5) New viruses infect other bacterial cells, continuing the cycle. **B**- Lysogenic Cycle: (1) Phage attaches and injects its DNA; (2) Viral DNA integrates into the bacterial genome as a prophage; (3) Prophage replicates along with the bacterial DNA during cell division; (4) Bacteria may gain new traits from the prophage. From [6]

1.2 Bacterial Defense Systems - R-M system

It has become evident that bacteria are constantly exposed to viral attacks. Over time, they have developed multiple defense mechanisms to defend themselves against these infectious agents [7] [8]. These defense mechanisms are crucial for bacterial survival in environments where viral infection is a continuous threat. One of the most studied defense systems is the restriction-modification (R-M) system, a form of innate immunity widely present in prokaryotes [9]. R-M systems safeguard bacterial cells by targeting and degrading foreign DNA, such as phage DNA, that invades cells. The main components of this mechanism include restriction enzyme (R), which cuts unmethylated DNA sequences, and methyltransferase (M), which protects the same DNA sequences [10]. When this system

is 'activated' the core enzymes will recognize and cut the exogenetic DNA, while, at the same time, they modify and protect the bacterial DNA [11], as represented in Figure 2. The R-M systems can be classified into four types, based on their components, sequence specificity, cofactors, and cleavage position [12].Type I-III R-M systems consist of genes that encode a restriction endonuclease (REase) and a methyltransferase (MTase), while Type IV R-M system includes only REase-related gene [8], as seen in Table 1.

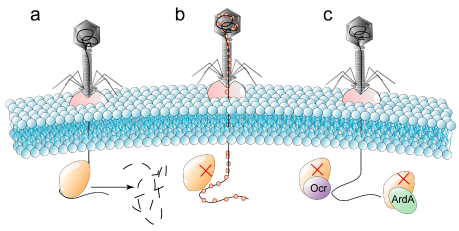

Fig. 2. The bacterial R-M system defends against phages by methylating its own DNA while cleaving unmethylated foreign DNA. (a) Unmethylated phage DNA is degraded by restriction endonucleases (REases). (b) Phages evade restriction by modifying their DNA. (c) Some phages produce anti-restriction proteins like Ocr and ArdA, which mimic DNA structure to inhibit REase activity, preventing cleavage. From [13]

Type I systems function as multifunctional complexes that both modify and restrict DNA, cutting at variable distances when ATP-dependent translocation is impeded [14, 15]. EcoKI in *Escherichia coli* exemplifies this system, requiring ATP, S-adenosylmethionine, and Mg^{2+} for restriction [16].

Type II systems are widely used in molecular biology due to their sequence-specific cleavage at fixed sites. They require Mg^{2+} as a cofactor and include variants such as Type IIS, which cut outside their recognition sites [15, 17]. In Helicobacter pylori, strain-specific variations mainly affect restriction genes, while methylation genes remain conserved [18].

Type III systems require two inversely oriented recognition sites for cleavage, using ATP-dependent translocation. The Mod subunit methylates adenines, preventing restriction if hemimethylation is present. Despite efficient activity *in vivo*, Type III enzymes show limited function *in vitro* [15, 19–22].

Type IV systems exclusively target modified DNA, such as methylated cytosines. *E. coli* EcoKMcrBC recognizes methylated sequences and cleaves near it, degrading phage DNA. This system blocks bacteriophage methyltransferases, triggering DNA fragmentation and host cell death [15, 23, 24].

1.3 Phage Evasion Strategies Against Bacterial Defense Systems

As bacteria have evolved defense mechanisms to protect themselves from viral infections, phages have simultaneously developed counter strategies to overcome

Feature	Type I	Type II	Type III	Type IV
		Structural		
Subunits	Three different	Two identical	Two different	Two different
Enzyme activity	Endonuclease, methyltransferase, ATPase	Endonuclease or methyltransferase	Endonuclease, methyltransferase, ATPase	Endonuclease, GTPase
		Biochemical		
Cofactors for DNA cleavage	ATP, AdoMet, Mg^{2+}	Mg^{2+}	ATP, Mg^{2+} (AdoMet)	Mg^{2+}, GTP
Methylation	AdoMet, Mg^{2+}	AdoMet	AdoMet, Mg^{2+}	—
Recognition sequence	Asymmetric, bipartite	Usually symmetric	Asymmetric, 25–27 bp from recognition site	Bipartite, methylated
Cleavage site	Random, at least 1000 bp from recognition site	At or near recognition site	25–27 bp from recognition site	Between methylated bases at multiple positions
DNA translocation	Yes	No	Yes	Yes

Table 1. Comparison of restriction-modification types. From [25]

these defenses [26]. As previously discussed, one key bacterial defense systems is the R-M system, which targets and cleaves foreign DNA. To evade this system, phages have developed two main resistance mechanisms: elimination of recognition sites in the viral genome and the production of anti-restriction proteins (Anti-REs) that actively inhibit restriction enzymes [27].

Phages have evolved strategies to evade bacterial R-M systems, one of which involves the methylation of their DNA to avoid recognition and cleavage by host restriction enzymes. The S-adenosylmethionine (SAM) lyase (SAMase) encoded by phage T3 plays a critical role in this process by degrading the SAM pool in Escherichia coli host cells. Since SAM is a key metabolite involved in multiple cellular functions, including DNA methylation, its reduction disrupts the host's ability to modify its own DNA and effectively neutralizes R-M system activity. The primary biological function of SAMase is to provide immunity to T3 phages against bacterial defenses [28].

Recent studies using single-particle cryo-electron microscopy and biochemical experiments have demonstrated that SAMase not only depletes SAM but also interacts with methionine S-adenosyltransferase (MAT), the enzyme responsible for SAM production. By inhibiting MAT, SAMase further reduces SAM availability in the bacterial cell, weakening the R-M system's ability to distinguish self from non-self DNA. Interestingly, phages lacking SAMase have been observed to evade host R-M systems just as effectively as those expressing the enzyme. While this suggests the presence of additional, yet unidentified, mechanisms that contribute to phage resistance, the precise details remain unclear. These findings highlight the complex interactions between phages and bacterial restriction-modification systems, emphasizing the ongoing evolutionary arms race between viral and bacterial defense mechanisms [29].

Another mechanism of resistance involves interfering with host DNA methylation processes, thereby preventing the activation of restriction enzymes and ensuring phage survival within the bacterial host [30]. A well-studied example is the coliphage T7 protein Ocr (overcome classical restriction), which structurally mimics DNA and inhibits the restriction complex. Another inhibitor, Stp from phage T4, binds type I restriction complexes such as EcoprrI, blocking restriction while leaving modification intact. However, this inhibition activates the bacterial anticodon nuclease PrrC, which cleaves $tRNA^{Lys}$, halting protein synthesis and serving as a secondary defense line [31] [32].

While some phages evade restriction through genome modification, bacteria have adapted by recognizing and cleaving modified DNA. These modification-dependent systems (MDSs), such as the type IV R-M system (e.g., GmrSD), target specific DNA modifications [33]. The T4 phage counters this with IPI*, an internal protein that binds GmrSD, preventing restriction [34] [35] [36] [37].

Not all phages rely on direct protein interactions to inhibit restriction enzymes. Some, like coliphage P1, encode DarA and DarB, which bind phage genomes and occlude restriction sites [38]. Others, such as coliphage λ proteins Ral and Lar, enhance bacterial methylation processes to shield their DNA from restriction enzymes [39] [40] [41].

1.4 Computational Approaches for Bacterial Defense Mechanisms

Machine learning is revolutionizing bacteriophage research by improving the identification of phage-related elements and enhancing our understanding of phage-bacteria interactions. Researchers are increasingly leveraging these models to tackle challenges such as detecting bacterial anti-phage defense mechanisms [42].

One such tool, DefenseFinder, detects prokaryotic antiviral systems, including R-M systems, with 91.9% sensitivity against the REBASE database. Despite its high accuracy, it underdetects approximately 15% of distant Type IV R-M systems, balancing sensitivity and specificity [43].

Another tool, PADLOC, is a web-based platform that analyzes bacterial and archaeal genomes to identify antiviral defense systems. Using a curated database of over 700 defense-related protein families and genetic synteny analysis, it effectively classifies multi-gene defense systems such as R-M systems [44].

The machine learning model DefensePredictor, developed by DeWeirdt et al., applies protein language model embeddings to classify defensive proteins. Tested on *E. coli* strains, it identified significantly more defense proteins than traditional methods, with 42% of predictions experimentally validated [42]. Although the article does not directly mention R-M systems, the use of protein language models may have the potential to identify phage evasion mechanisms that bypass these defenses. Therefore, even if the model was not designated for this purpose, this approach could represent a promising strategy for application to our problem.

Together, these studies highlight the role of machine learning in bacteriophage research, accelerating genome annotation, uncovering new therapeutic

targets, and deepening our understanding of phage-bacteria interactions [42]. While machine learning has already been applied to predict bacterial defense systems, its potential to identify phage-encoded anti-restriction mechanisms remains largely unexplored.

2 WorkPlan

This project aims to investigate the interaction between phages and bacterial R-M systems, focusing on how phages evade these defense mechanisms. To achieve this, computational approaches, including Python-based tools and machine learning (ML) models, will be employed. The specific objectives are:

1. Conduct a literature review on bacterial R-M systems and phage evasion strategies by exploring studies that reference the classification and mechanisms of the systems, as well as the phage evasion strategies. Focusing on experimental evidence, and recent findings.

2. A curated dataset of phage-host pairs will be constructed based on the literature review. This dataset will include: (a) Host species: Identification of bacterial species targeted by specific phages (with a focus on selected bacterial species of interest); (b) Phage species (documenting phages known to infect these hosts); (c) Anti-restriction proteins: Documentation of proteins involved in evading R-M systems. This dataset will ensure that all relevant information is systematically organized for easy access and analysis.

3. A comprehensive script will be created to extract relevant data from the REBASE database, focusing on key enzyme attributes such as recognition sequences, cleavage sites, and the corresponding bacterial hosts. This information will be used to assess the presence of specific cleavage sites within phage genomes. Using these results, we will identify avoidance patterns, analyzing how certain phages evade restriction endonucleases (REases), potentially through sequence variation or other genomic adaptations.

4. Machine learning models will be developed to predict anti-restriction proteins encoded in phage genomes. The approach includes: (a) Data preprocessing: Leveraging the curated dbAPIS dataset to extract relevant information, including anti-restriction protein sequences and also phage-host interaction metadata; (b) Model development: Applying and optimizing machine learning algorithms tailored for biological sequence analysis (c) The models will be trained to predict anti-restriction functions based on protein-derived features extracted from phage-encoded proteins. These features will be integrated with phage-host interaction data and bacterial R-M system profiles to capture functional signatures associated with phage evasion mechanisms.

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