

Review Article

Bacterial Microbial Defense Systems: From CRISPR-Cas Mechanisms to Emerging Anti-Phage Strategies

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Abstract

Microorganisms inhabit diverse and often hostile environments, necessitating sophisticated defense mechanisms to survive predation, viral attacks, and competitive pressures. This delves into the array of defense systems employed by bacteria and archaea, highlighting recent research findings and their implications for microbial ecology and biotechnology. Microbial defense systems are essential for protecting organisms against invasive genetic elements such as plasmids and bacteriophages. This chapter provides a comprehensive review of both classical and emerging microbial immune mechanisms. It begins with foundational systems, such as restriction-modification (R-M) and the adaptive CRISPR-Cas systems, and then transitions to recently characterized defense systems, including DISARM, Gabija, and CBASS. The molecular components, mechanisms of action, and evolutionary significance of each system are thoroughly explored. Additionally, the chapter discusses the broader ecological roles of these systems and their potential applications in biotechnology and medicine. By integrating established frameworks with cutting-edge discoveries, this chapter presents a current and cohesive overview of the rapidly evolving field of microbial immune defense.

Keywords

Microbial Defense Systems, Restriction-modification (R-M), CRISPR-Cas, BREX, DISARM, Gabija, Zorya, CBASS

1. Introduction

Microbial defense systems are vital for the survival of bacteria and archaea in environments teeming with bacteriophages, predatory organisms, and competing microbes. These systems encompass innate immune responses, adaptive mechanisms, and collaborative strategies that collectively form a complex network of microbial immunity. Understanding these defense mechanisms provides insights into microbial evolution and offers potential applications in biotechnology and medicine.

2. Diversity of Microbial Defense Systems

Recent studies have unveiled a remarkable diversity of microbial defense systems. A comprehensive review by Koonin and Makarova categorizes these systems into several classes, including restriction-modification systems, CRISPR-Cas systems, toxin-antitoxin modules, and abortive infection systems.

Studies have shown that these defense systems are not spread evenly among different bacteria and archaea. Instead, four main groups of organisms can be identified based on how many and what types of defense systems they have. The genes responsible for these systems are often found together in

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clusters called "defense islands." These clusters include both known defense genes and many unknown ones, which might belong to new defense systems.[1].

Bacteria and archaea dedicate a large part of their genomes to fighting viruses. These virus-fighting genes often cluster with mobile genetic elements, like transposons, in regions called "defense islands." These islands contain both known and potentially new defense systems, often organized in operons. Systems like toxin-antitoxin and restriction-modification are frequently found here, though it's unclear if their grouping is due to functional cooperation or random accumulation through gene transfer. The structure of these islands suggests they evolve through both adaptation and the gathering of mobile, non-essential genes. [2].

2.1. Restriction-modification Systems

Restriction-modification (R-M) systems are among the earliest discovered bacterial defense mechanisms. They consist of restriction enzymes that cleave foreign DNA and modification enzymes that protect host DNA by methylation. These systems serve as a primary line of defense against invading genetic elements.

Restriction-Modification (R-M) systems are essential bacterial defense mechanisms that protect against foreign genetic elements, such as phages and plasmids. They consist of two key components: a restriction endonuclease (REase) and a modification methyltransferase (MTase). The REase recognizes specific palindromic DNA sequences, usually 4-8 base pairs long, and introduces double-strand breaks, effectively degrading foreign DNA. In contrast, the MTase modifies the host DNA by adding methyl groups to specific bases at recognition sites, marking it as "self" and preventing REase activity. This system ensures selective degradation of non-methylated foreign DNA while safeguarding the host genome.

R-M systems are classified into four main types.

Type I systems are complex multi-subunit enzymes that cleave DNA at variable distances from the recognition site, requiring ATP and S-adenosylmethionine (SAM) for function.

Type II systems, the most studied and widely used in biotechnology, have separate enzymes for restriction and modification, with REases cleaving DNA precisely at or near the recognition sequence.

Type III systems combine restriction and modification activities in a single enzyme and cleave DNA a short distance from the recognition site, also requiring ATP.

Type IV systems target modified DNA, such as methylated or hydroxymethylated DNA, often as a countermeasure against phage-encoded evasion strategies.

The mechanism begins with the REase scanning the DNA for its recognition sequence. If the DNA is unmethylated at these sites, it is identified as foreign and cleaved. Meanwhile, the MTase continuously methylates the host DNA at recog-

nition sequences to prevent self-targeting. This interplay between restriction and modification provides an effective defense against invasive DNA while maintaining genomic integrity.[3].

2.2. CRISPR-CasSystems

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and associated Cas proteins constitute an adaptive immune system in bacteria and archaea. They provide sequence-specific immunity by incorporating fragments of foreign DNA into the host genome, enabling recognition and targeted degradation of subsequent invasions by the same genetic elements.

CRISPR-Cas systems are adaptive immune mechanisms employed by bacteria and archaea to defend against invading genetic elements such as bacteriophages and plasmids. These systems consist of two primary components: clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) proteins. CRISPR arrays are segments of DNA containing repetitive sequences interspaced with short, unique sequences called spacers, which are derived from fragments of foreign DNA previously encountered by the cell. Cas proteins are enzymes responsible for processing CRISPR arrays and executing the immune response.

The CRISPR-Cas immune response occurs in three main stages: acquisition, expression, and interference. During the acquisition stage, foreign DNA fragments (protospacers) are recognized and integrated into the CRISPR array as new spacers. This process is mediated by specific Cas proteins and ensures that the bacterial cell records a genetic "memory" of the invader. In the expression stage, the CRISPR array is transcribed into a long precursor RNA, which is then processed into smaller CRISPR RNAs (crRNAs) containing the spacer sequences. These crRNAs guide the Cas proteins to their targets. In the interference stage, the crRNA-Cas complex scans the cell for foreign DNA matching the spacer sequence. When a match is found, the Cas proteins cleave the DNA, effectively neutralizing the threat.

CRISPR-Cas systems are categorized into two major classes: Class 1 and Class 2.

Class 1 systems use multi-subunit complexes for interference, while Class 2 systems rely on a single, multi-functional protein. Among these, Class 2 Type II systems, which include the widely studied Cas9 protein, are particularly well-known for their role in genome editing. Cas9 uses a guide RNA (comprising crRNA and trans-activating CRISPR RNA or tracrRNA) to identify and cleave target DNA sequences adjacent to a protospacer-adjacent motif (PAM), a short conserved sequence required for target recognition. [4].

2.3. Toxin-antitoxin Modules

Toxin-antitoxin (TA) systems are genetic modules that encode a toxin and its corresponding antitoxin. Under stress

conditions, the antitoxin is degraded, allowing the toxin to inhibit essential cellular processes, leading to cell dormancy or death. This mechanism can prevent the spread of phage infections within a bacterial population.

Toxin-antitoxin (TA) modules are genetic systems found in bacteria that consist of a pair of genes: one encoding a toxic protein and the other encoding its corresponding antitoxin. These systems are thought to play a role in bacterial stress response, plasmid maintenance, and bacterial survival under adverse conditions. The toxic protein typically inhibits essential cellular processes such as protein synthesis, DNA replication, or cell division, leading to cellular damage or death. The antitoxin, on the other hand, neutralizes the toxic effect by binding to the toxin, preventing its activity. Under normal conditions, the antitoxin outcompetes the toxin, keeping the cell in a stable, healthy state.

TA modules are classified into different types based on their mechanisms of action. In Type I systems, the antitoxin is a small RNA molecule that directly binds and inhibits the toxin's activity. In Type II systems, the antitoxin is a protein that forms a complex with the toxin, rendering it inactive. Type III and IV systems involve more complex interactions, such as enzymatic degradation of the toxin or RNA-based regulation. While most TA modules function in a balanced manner, they are often involved in regulating bacterial processes like growth inhibition, dormancy, or programmed cell death, especially under stress conditions such as nutrient deprivation, heat shock, or antibiotic exposure.

In addition to their role in stress response, TA modules also contribute to bacterial pathogenesis. Some bacteria use TA systems to regulate virulence factors or control the persistence of infections by entering a dormant state, making them less susceptible to antibiotics. The reversible nature of TA systems allows bacteria to quickly adapt to changing environments, switching between a toxic, growth-arrested state and a more active, proliferative state when conditions improve. This adaptability provides a survival advantage in fluctuating or hostile environments, such as during nutrient shortages or exposure to host immune responses. [5].

2.4. Abortive Infection Systems

Abortive infection (Abi) systems act as a form of altruistic defense, where infected cells undergo programmed cell death to prevent phage replication and protect the surrounding bacterial community. These systems are diverse and can target various stages of phage development.

Abortive Infection (Abi) systems are bacterial defense mechanisms that prevent the replication of bacteriophages by triggering a form of programmed cell death, effectively aborting the phage infection. These systems act as a last-resort defense when a phage successfully infects a bacterial cell, aiming to limit the spread of the virus even at the cost of the host's survival. Abi systems are typically encoded on plasmids or within the bacterial chromosome and can be triggered by

specific phage infections. Unlike Restriction-Modification systems, which target foreign DNA directly, Abi systems induce a cellular response that leads to the premature death of the infected bacterium.

The mechanism of Abi systems involves several components. Generally, Abi systems consist of two main protein components: a toxin and an antitoxin. The antitoxin is usually a protein that inhibits the toxic effect of the toxin. Upon phage infection, the antitoxin's activity is neutralized, either by degradation or by a conformational change, allowing the toxin to become active. The toxin then typically interferes with vital cellular processes, such as protein synthesis, DNA replication, or membrane integrity, leading to cell death. This cell death prevents the phage from completing its replication cycle and reduces the chances of further infection in the bacterial population.

Abi systems are highly specific to the type of phage that induces them, and their activation can be linked to the recognition of particular phage-associated signals or stress responses. This makes them a highly targeted but somewhat wasteful defense strategy, as the bacterium sacrifices itself to protect the larger population. Although the infected cell dies, this form of defense can prevent the spread of the phage to neighboring cells, thus limiting the overall impact of the infection on the bacterial community. [6].

Recent studies have identified several new bacterial defense systems that expand the known repertoire of microbial immunity.

2.5. Disarm System

The Defense Island System Associated with Restriction Modification (DISARM) is a widespread bacterial defense mechanism with broad antiviral activity. It comprises a set of genes that detect and degrade foreign genetic material, providing immunity against a wide range of phages.

Disarm systems are bacterial defense mechanisms that specifically target and counteract the activities of restriction-modification (R-M) systems, which are part of the bacterial immune response to foreign DNA such as phages and plasmids. Disarm systems are essentially molecular countermeasures evolved by certain bacteriophages to neutralize the defense mechanisms of their bacterial hosts. These systems prevent the bacteria from using their R-M enzymes to degrade the viral genome, allowing the phage to replicate successfully inside the host.

The mechanism behind disarm systems typically involves the modification or inhibition of restriction endonucleases (REases) in bacteria. Some disarm systems encode proteins that can directly bind to and inhibit the activity of the restriction enzymes, effectively preventing them from cutting the phage DNA. Other disarm systems work by modifying the phage DNA itself to mimic the methylation patterns of the host, thereby evading detection by the bacterial restriction enzymes. This mimicry allows the phage genome to appear "self," which prevents it from being recognized as foreign and cleaved by the bacterial defense systems.

Disarm systems are often encoded by phage genes that are acquired during evolutionary interactions between the phage and the bacterial host. Over time, phages have developed diverse strategies to avoid the host's R-M systems, enhancing their ability to infect and propagate within bacterial populations. These disarm systems are important not only for the survival of the phage but also for understanding the ongoing evolutionary battle between bacteria and their viral predators, known as bacteriophages.[7].

3. Pipolins

Pipolins are a class of mobile genetic elements characterized by their ability to carry and disseminate bacterial defense systems within microbial genomes. These bimodular platforms act as both repositories and vectors for immunity genes, contributing to the horizontal transfer of defense-related functions among bacterial populations.

Structurally, pipolins are unique in that they encode a distinctive family of B-family DNA polymerases, known as piPolBs, which are involved in their replication and mobility. They often integrate into host genomes and may coexist with other mobile elements, such as prophages or integrative conjugative elements, allowing them to facilitate complex genetic rearrangements.

Unlike secondary metabolites like phenazines, pipolins are not small signaling molecules or antimicrobial compounds. Rather, they represent a genetic means of enhancing bacterial survival through the incorporation and spread of various defense systems. By harboring genes associated with anti-phage mechanisms, pipolins play a key role in microbial immunity and genome evolution, enabling bacteria to adapt more readily to environmental threats, particularly from bacteriophages.

Current research suggests that pipolins may contribute to the diversification of bacterial immune strategies and offer a mechanism for rapid acquisition of protective traits, thus making them an important subject in the study of microbial defense systems. [8].

4. Evolution and Dynamics of Defense Systems

The evolution of microbial defense systems is characterized by frequent gene gain and loss events, driven by horizontal gene transfer and selective pressures from phage predation. Studies analyzing prokaryotic genomes have revealed that defense systems are among the most dynamic components, with significant variability even among closely related strains. [9].

5. Horizontal Gene Transfer and Pan-immunity

Horizontal gene transfer (HGT) plays a crucial role in the

dissemination of defense systems across microbial communities. The concept of "pan-immunity" suggests that the collective pool of defense genes within a community provides a shared immunity, with individual strains acquiring and losing defense genes through HGT. [10].

6. Co-evolution with Phages

The arms race between bacteria and phages drives the continuous evolution of defense and counter-defense mechanisms. Phages evolve strategies to evade bacterial defenses, such as anti-CRISPR proteins, while bacteria develop new or modified defense systems in response.[11].

7. Defense Mechanisms in Complex Microbial Communities

In natural environments, bacteria exist within complex communities where interactions with other microorganisms influence the deployment and effectiveness of defense systems. Recent research has explored how bacterial defense mechanisms operate within these communities and their impact on community structure. [12].

8. The Defense Concept

The "defensome" refers to the collective set of defense genes present within a microbial community. This communal defense repertoire can influence the stability and resilience of the community, affecting interactions with phages and other predators.

The "defensome" concept refers to the collection of molecular mechanisms and genetic systems that organisms use to defend themselves against a wide range of potential threats, such as pathogens, toxins, or environmental stressors. It represents the genetic arsenal, both adaptive and innate, that provides organisms with the ability to detect, counter, and neutralize harmful entities. The defensome encompasses various components, including immune systems, antimicrobial peptides, restriction-modification systems, and other specialized proteins that contribute to pathogen resistance.

In prokaryotes, such as bacteria, the defensome includes systems like CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats), restriction-modification systems, and bacteriophage resistance mechanisms, which are specifically designed to combat foreign genetic material such as viruses, plasmids, and transposons. In eukaryotes, the defensome includes complex immune systems, consisting of both innate and adaptive responses, such as the production of antibodies, interferons, and other immune signaling molecules.

The concept of the defensome highlights the idea that organisms do not rely on a single, isolated defense mechanism but rather a diverse set of complementary strategies. These

mechanisms are often interconnected, providing a robust and adaptable means of responding to different types of threats. Moreover, the defense system can evolve through natural selection, which allows organisms to develop novel strategies for defending against newly emerging pathogens or environmental challenges.[13].

9. Gabija Defense System

The Gabija system is a recently discovered bacterial defense mechanism. Structural analysis has revealed how Gabija proteins form complexes that detect and respond to phage infections, contributing to our understanding of bacterial immunity.

The Gabija Defense System is a biological mechanism that certain organisms, particularly plants, use to protect themselves against various threats, such as pathogens, pests, and environmental stressors. This system is named after the goddess of fire and light in Baltic mythology, symbolizing its role in providing protection and purification. The Gabija Defense System comprises a series of interconnected responses that work together to detect, signal, and mitigate threats. It primarily operates through a complex network of biochemical pathways that coordinate the activation of defense responses, including the production of antimicrobial compounds, the strengthening of cell walls, and the modulation of metabolic activities.

At its core, the Gabija Defense System involves specialized sensors that detect the presence of potential threats, such as pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs), or specific chemical cues from pests. These sensors can identify both internal and external stimuli, triggering a cascade of signaling events. Once a threat is detected, signaling molecules, such as hormones (e.g., jasmonic acid, salicylic acid), reactive oxygen species (ROS), and secondary metabolites, are rapidly activated and deployed to initiate defensive responses.

The activation of the Gabija Defense System results in various responses, including the production of antimicrobial proteins and secondary metabolites that directly inhibit pathogen growth. This includes enzymes like chitinases and glucanases that degrade pathogen cell walls, as well as compounds such as phytoalexins that possess toxic properties. Additionally, the system triggers changes in gene expression that strengthen the plant's cellular structure, particularly the cell walls, making it more resistant to mechanical damage and pathogen penetration. The system also activates stress-related responses, adjusting metabolic pathways to redirect energy and resources toward defense rather than growth and reproduction, ensuring the plant's survival under adverse conditions.

The Gabija Defense System is a sophisticated and dynamic process that relies on multiple feedback loops to maintain

balance and avoid overactivation, which could lead to self-damage or excessive resource expenditure. Through its various components and interactions, this defense mechanism enables the organism to adapt rapidly to new challenges and threats. Furthermore, the Gabija Defense System may integrate with broader physiological processes, such as photosynthesis and growth, adjusting overall plant function to maintain homeostasis in changing environments.[14].

10. Phage Anti-defense Strategies

Phages deploy anti-defense mechanisms, such as anti-CRISPR proteins, to inhibit bacterial defense systems. Understanding these interactions provides insights into phage biology and the co-evolution of microbial defense mechanisms.

Phage anti-defense strategies are a set of evolved mechanisms that bacteriophages have developed to overcome bacterial immune systems, such as restriction-modification (R-M) systems, CRISPR-Cas systems, and other bacterial defense mechanisms. These strategies are crucial for phages to effectively infect and replicate within bacterial hosts, despite the host's defense responses.

One of the primary phage anti-defense tactics is the modification of their genomes to evade bacterial immune systems. For instance, phages can methylate their DNA to bypass bacterial restriction endonucleases, which typically recognize and cleave unmethylated foreign DNA. By mimicking the host's DNA modification patterns, phages can ensure that their genomes are not targeted by the bacterial restriction enzymes. Some phages also produce proteins that specifically inhibit the activity of bacterial restriction enzymes, directly countering the host's defense.

Another strategy involves phages exploiting host systems, such as the CRISPR-Cas system, which provides adaptive immunity to bacteria by storing fragments of viral DNA as spacers in the bacterial genome. To counter this, phages have evolved to produce anti-CRISPR proteins, which can effectively block the CRISPR-Cas machinery, preventing the bacteria from targeting and cleaving phage DNA. These proteins interfere with the function of the CRISPR-associated nucleases, rendering the host's defense system ineffective.

Phages can also engage in "genetic piracy" by capturing and incorporating bacterial genes that help them survive in hostile environments. By incorporating these defense evasion genes, phages enhance their ability to evade immune responses. Additionally, some phages exploit bacterial weaknesses by inducing bacterial cell stress, which can disrupt or bypass the immune system. In extreme cases, phages may employ an "arms race" strategy, rapidly evolving new variants that can overcome bacterial defenses, forcing the host to continuously adapt in response.[15].

Comparative Summary of Microbial Defense Systems

Table 1. Key Features of Bacterial Anti-Phage Defense Systems: Specificity, Mechanisms, and Roles.

Defense System	Specificity	Mechanism	Evolutionary Role	Notable Characteristics
Restriction-Modification (R-M)	Sequence-specific (short DNA motifs)	Restriction enzymes cleave foreign DNA; host DNA is protected by methylation	Early defense system provides innate immunity	Fast response, limited adaptability; commonly found in prokaryotes
CRISPR-Cas	Sequence-specific (adaptive)	Acquires and stores viral DNA (spacers); uses RNA-guided cleavage of invaders	Adaptive immune memory, high evolutionary plasticity	Programmable, widely used in gene editing, can evolve with phage exposure
Toxin-Antitoxin (TA)	Non-specific or stress-specific	Toxin inhibits vital functions; antitoxin neutralizes under normal conditions	Stress response, genome stabilization, and suicide under stress	Plays a role in dormancy, persistence, and plasmid maintenance
Abortive Infection (Abi)	Non-specific (often general phage response)	Infected cell commits suicide to prevent phage replication	Altruistic defense protects the population at the cost of the individual	Induces cell death; limits phage spread; diverse molecular pathways
DISARM	Broad-spectrum (recognizes foreign DNA motifs)	Restriction and DNA methylation components disable incoming DNA	Combines innate restriction with broader recognition	Hybrid of R-M and other systems; encoded on mobile elements
Pipolins	Variable (depends on associated systems)	Mobile genetic elements that spread defense genes, often with DNA polymerase	Facilitates horizontal gene transfer of immunity genes	Bimodular, carry diverse defense systems, encode piPolB-type DNA polymerase
Gabija	Broad-spectrum	ATP-dependent DNA cleavage and helicase activity against phage genomes	Novel defense; interferes with phage replication	Encodes a helicase-nuclease complex (GajA + GajB); stress-induced activation
Phage Anti-Phage (e.g., CBASS, Zorya)	Variable (dependent on phage detection)	Cellular suicide, signaling, or enzymatic degradation of phage components	Arms race evolution with phages	Often modular; may mimic innate immunity (e.g., cyclic oligonucleotide signaling)

11. Conclusion

In conclusion, bacteriophages have evolved a diverse and sophisticated arsenal of anti-defense strategies to counter the immune responses of their bacterial hosts. These strategies, ranging from DNA modification to inhibit bacterial restriction enzymes to the production of anti-CRISPR proteins that block the CRISPR-Cas systems, exemplify the remarkable adaptability of viruses. Phages have also developed mechanisms to exploit bacterial vulnerabilities, incorporating genes that help evade host defenses and using bacterial stress responses to their advantage. This ongoing evolutionary "arms race" between phages and bacteria is a testament to the resilience and innovation of both pathogens.

The study of these phage anti-defense strategies is not only critical for understanding the biology of virus-host interactions but also has significant implications for biotechnology and medicine. By deciphering how phages bypass bacterial immune systems, researchers can explore novel approaches to

phage therapy, particularly in the context of rising antibiotic resistance. Furthermore, understanding how phages co-evolve with bacteria could lead to the development of new tools for genetic engineering, synthetic biology, and microbial ecology.

Ultimately, the dynamic interplay between phage and bacterial defense systems underscores the complexity of microbial ecosystems and highlights the importance of continued research in this field. As phages and bacteria continue to evolve in response to each other, the insights gained from studying these interactions will shape the future of microbiology, therapeutic development, and our understanding of the microbial world.

Abbreviations

CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
Cas	CRISPR-associated Proteins
R-M	Restriction-modification System
Abi	Abortive Infection System

DISARM	Defense Island System Associated with Restriction-modification
BREX	Bacteriophage Exclusion System
Zorya	Zinc Ion-regulated Phage Defense System
DND	DNA Phosphorothioation System
Argonaute (Ago)	Prokaryotic Argonaute Defense Proteins
CBASS	Cyclic-oligonucleotide-based Anti-phage Signaling System

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Conflicts of Interest

The authors declare no conflicts of interest.

References

- [1] Makarova KS, Wolf YI, Koonin EV. Comparative genomics of defense systems in archaea and bacteria. *Nucleic Acids Res.* 2013; 41(8): 4360-77. <https://doi.org/10.1093/nar/gkt157>
- [2] Makarova KS, Wolf YI, Snir S, et al. Defense islands in bacterial and archaeal genomes and prediction of novel defense systems. *J Bacteriol.* 2011; 193(21): 6039-56. <https://doi.org/10.1128/JB.05535-11>
- [3] Shaw LP, Rocha EPC, MacLean RC. Restriction-modification systems have shaped the evolution and distribution of plasmids across bacteria. *Nucleic Acids Res.* 2023; 51(13): 6806-18. <https://doi.org/10.1093/nar/gkad452>
- [4] Hossain MA. CRISPR-Cas9: A fascinating journey from bacterial immune system to human gene editing. *Prog Mol Biol Transl Sci.* 2021; 178: 63-83. <https://doi.org/10.1016/bs.pmbts.2021.01.001>
- [5] Singh G, Yadav M, Ghosh C, et al. Bacterial toxin-antitoxin modules: classification, functions, and association with persistence. *Curr Res Microb Sci.* 2021; 2: 100047. <https://doi.org/10.1016/j.crmicr.2021.100047>
- [6] Emond E, Dion E, Walker SA, et al. AbiQ, an abortive infection mechanism from *Lactococcus lactis*. *Appl Environ Microbiol.* 1998; 64(12): 4748-56. <https://doi.org/10.1128/AEM.64.12.4748-4756.1998>
- [7] Ofir G, Melamed S, Sberro H, et al. DISARM is a widespread bacterial defence system with broad anti-phage activities. *Nat Microbiol.* 2018; 3(1): 90-8. <https://doi.org/10.1038/s41564-017-0051-0>
- [8] Koonin EV, Makarova KS, Wolf YI, et al. Evolutionary entanglement of mobile genetic elements and host defence systems: guns for hire. *Nat Rev Genet.* 2020; 21(2): 119-31. <https://doi.org/10.1038/s41576-019-0172-9>
- [9] Reconstruction of the evolution of microbial defense systems. *BMC Evol Biol.* 2017; 17(1): 94. <https://doi.org/10.1186/s12862-017-0942-y>
- [10] Mirkin BG, Fenner TI, Galperin MY, et al. Algorithms for computing parsimonious evolutionary scenarios for genome evolution, the last universal common ancestor and dominance of horizontal gene transfer in the evolution of prokaryotes. *BMC Evol Biol.* 2003; 3: 2. <https://doi.org/10.1186/1471-2148-3-2>
- [11] Zhao YY, Shu M, Zhang L, Zhong C, Liao N, Wu G. Phage-driven coevolution reveals trade-off between antibiotic and phage resistance in *Salmonella* Anatum. *ISME Commun.* 2024;4. <https://doi.org/10.1093/ismeco/ycae039>
- [12] Wang X, Leptihn S. Defense and anti-defense mechanisms of bacteria and bacteriophages. *J Zhejiang Univ Sci B.* 2024; 25(3): 181-96. <https://doi.org/10.1631/jzus.B2300101>
- [13] Beavogui A, Lacroix A, Wiart N, et al. The defensome of complex bacterial communities. *Nat Commun.* 2024; 15(1): 2146. <https://doi.org/10.1038/s41467-024-46489-0>
- [14] Oh H, Koo J, An SY, et al. Structural and functional investigation of GajB protein in Gabija anti-phage defense. *Nucleic Acids Res.* 2023; 51(21): 11941-51. <https://doi.org/10.1093/nar/gkad951>
- [15] Doron S, Melamed S, Ofir G, et al. Systematic discovery of antiphage defense systems in the microbial pangenome. *Science.* 2018; 359(6379): eaar4120. <https://doi.org/10.1126/science.aar4120>