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ANTARCTIC BACTERIOPHAGES: THEIR ECOLOGICAL ROLE AND BIOTECHNOLOGICAL POTENTIAL

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ABSTRACT

Abstract
Viruses that infect bacteria (bacteriophages or phages) are the most abundant biological entities in the biosphere. Phages play a central role in shaping microbial communities and regulating global biogeochemical cycles. Two major biological cycles characterize phages: the lytic and the lysogenic cycles. This study focuses on the lysogenic cycle, in which phages integrate into the bacterial genome. The study of bacterium–phage systems in Antarctic environments is of particular interest due to the extreme environmental conditions under which these interactions occur. Antarctic bacteria isolated from different habitats were investigated to characterize their lysogenic viruses. Bizionia argentinensis, Rhodococcus spp. strain ADH, and Agreia spp. were characterized both in silico and in vitro for the presence of integrated phages (prophages). These prophages harbor a genetic repertoire that may play a central role in host–environment interactions. In addition, some prophage-derived genes (including endolysins, enzymes that lyse bacterial cell walls) have potential biotechnological applications. In particular, enzymes adapted to function at low temperatures could be exploited for industrial use.

KEY WORDS

Evolution; Bacteriophages, Lysogeny; Genes of interest, Biotechnology, Antarctica, Antarctic Treaty, Antarctic Environmental Protection, Antarctic Tourism

INTRODUCTION

Antarctica represents one of the most extreme and least explored ecosystems on Earth, characterized by unique environmental conditions that pose constant challenges to life. In this hostile environment, microbial communities play critical roles in global biogeochemical cycles and in maintaining ecosystem functioning. Among these microorganisms, bacteriophages emerge as key regulators of microbial dynamics, exerting a decisive influence on the structure of bacterial communities and on the flow of matter and energy.

What are bacteriophages and what are their main biological characteristics?

Bacteriophages, or simply phages, are viruses that infect bacteria. Like any virus, they depend on the cellular machinery of their host to reproduce. They are considered the most diverse and abundant biological entities in the biosphere (Suttle 2007; Anderson et al. 2011; Paez-Espino et al. 2016; Carroll et al. 2018). It is estimated that there are around 10^{31} bacteriophage particles in nature, and approximately 10^{23} phage infections occur every second worldwide (Weitz and Wilhelm 2012). This highlights not only the magnitude of their population but also their dynamics and relevance in various fields. Bacteriophages play a crucial role in several processes: a) Global ecology: they regulate microbial populations and affect carbon and nutrient flows in ecosystems; b) Microbial evolution: they promote the diversification of microorganisms and facilitate horizontal gene transfer; c) Scientific research: They have been key tools in molecular biology and in the study of cells at the molecular level; d) Healthcare system: They are being investigated as alternatives for the control of antibiotic-resistant bacterial infections (Weitz and Wilhelm 2012; Jamal et al. 2019; Kim et al. 2020). Bacteriophages are classified according to the type of genome they possess, which can be either DNA (deoxyribonucleic acid) or RNA (ribonucleic acid). Most bacteriophages have double-stranded DNA (dsDNA). However, there are also phages with single-stranded DNA (ssDNA), single-stranded RNA (ssRNA), or double-stranded RNA (dsRNA). They exhibit a wide variety of shapes and structures. Most lack a lipid envelope, although some have an additional lipid layer. In terms of shape, we find tailless phages and phages with head-tail structures, which are the most common. These latter ones can have long, contractile tails, long, non-contractile tails, or short, non-contractile tails. (Figure 1).

One of the most fascinating findings from comparative studies of bacteriophage genomes is their mosaic nature (Gauthier and Hatfull 2024), meaning they have DNA segments from different origins in their genome. This phenomenon is mainly explained by horizontal gene transfer, where bacteriophages share their genetic material with other organisms, resulting in genomes with a unique mix of genes and contributing significantly to their evolution and diversity (Hatfull and Hendrix, 2011).

Bacteriophage life cycles

Bacteriophages have two main strategies for multiplying: the lytic cycle and the lysogenic cycle (Mavrich and Hatfull 2017). In the lytic cycle, the phage infects the bacterium and uses its cellular machinery to replicate its own genome and produce new viral particles. At the end of this process, the bacterial cell ruptures (lysis), releasing the new phages that can infect other evolutionarily related bacteria. This cycle is characteristic of virulent phages, which cause lysis and kill the host cell. In

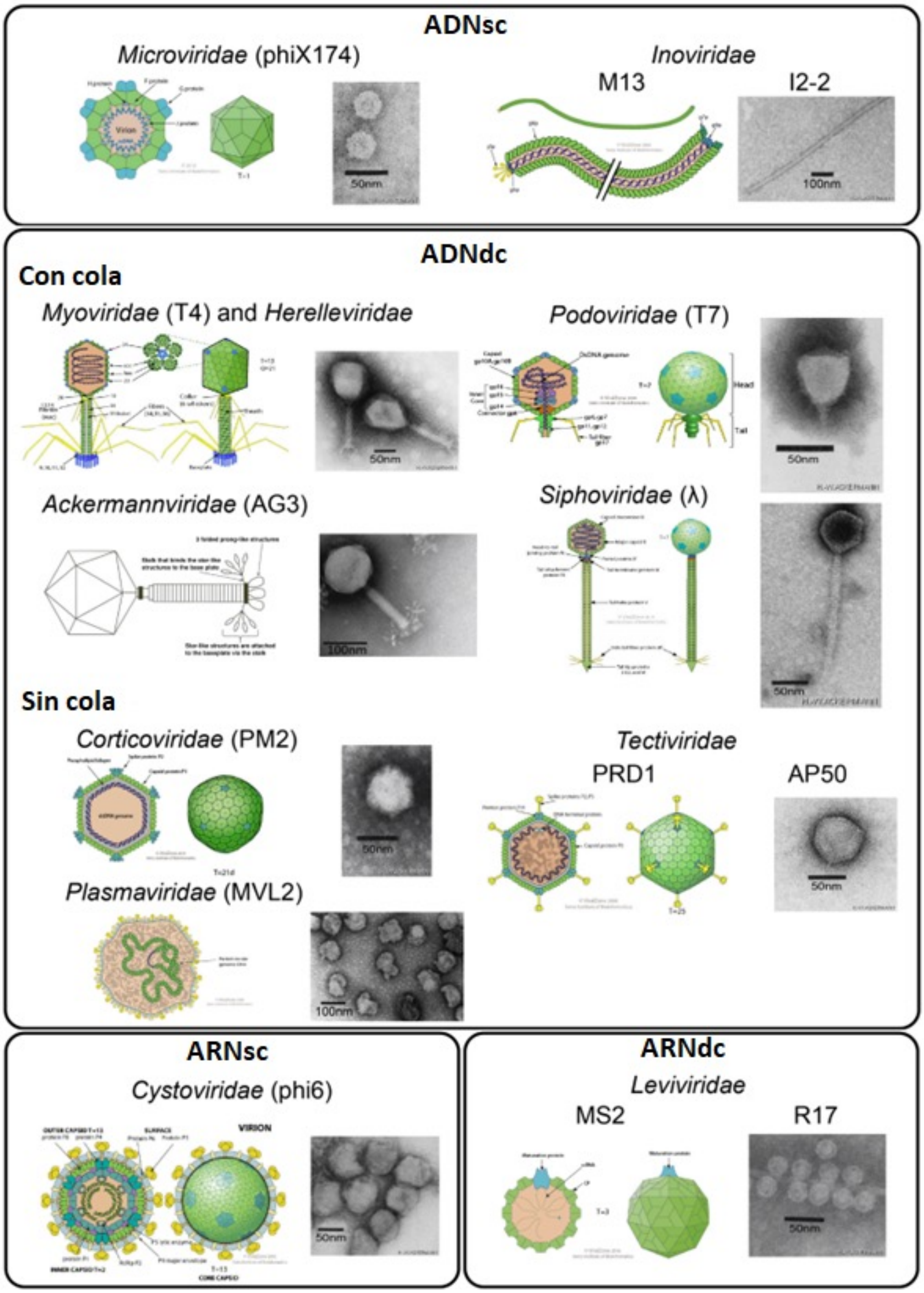

Figure 1. *Classification of bacteriophages based on genome type and morphology. The bar indicates the size scale (Dion et al. 2020).*

the lysogenic cycle, after infection, the phage genome integrates into the bacterial DNA. In its form integrated into the bacterial chromosome, the phage is identified as a prophage. At this stage, the viral genome can be transmitted vertically (from parent cells to daughter cells) to subsequent generations of the bacterium. Under certain environmental conditions, which can stress the bacterial cell, the prophage can be activated, detach from the bacterial genome, and restart the lytic cycle. This process is known as induction and marks the beginning of the lytic cycle. Phages that can alternate between both cycles are called temperate phages. Lysogeny is a process in which the bacteriophage genome integrates into the host bacterium's genome, allowing their coexistence without destroying the bacterial cell. This state offers significant advantages for both the virus and the bacterium. On the one hand, the phage ensures its survival under adverse conditions, such as when there is a low density of susceptible bacteria, and guarantees its propagation through vertical replication along with the bacterial DNA. On the other hand, the bacterium can benefit from lysogenic conversion, acquiring new characteristics derived from the prophage genes. These can include genes that encode toxins, such as the cholera toxin of Vibrio cholerae, the diphtheria toxin of Corynebacterium diphtheriae, or the Shiga toxin of Escherichia coli O157:H7 (all examples of great clinical and pathological significance). Furthermore, prophage genes can confer antibiotic resistance or modify bacterial structures, such as the cell wall or surface proteins, making the bacteria less detectable by the immune system of humans or animals during an infection or more resistant to other phages. They function as a reservoir of mobile genes, facilitating their horizontal transfer and promoting microbial evolution.

Bacterial defense systems against bacteriophage infection

Bacteria have developed a variety of defense systems to protect themselves from bacteriophage infection, including physical, chemical, and genetic mechanisms. These systems represent a kind of "evolutionary arms race" between bacteria and phages. The main bacterial defense mechanisms are described below: a) physical barriers such as the modification of surface receptors; b) innate immune systems such as the DNA Restriction and Modification system or the Toxin-Antitoxin system; c) adaptive immune systems such as the CRISPR-Cas system; d) abortive defense systems such as the Abi system (Wang et al. 2010; Makarova et al. 2011; Shi et al. 2020; Payne et al. 2021; Tesson et al. 2024).

Although bacteria have multiple defense mechanisms to protect themselves from bacteriophages, these viruses sometimes manage to inject their genetic material into them. This occurs thanks to the ability of phages to evolve and adapt, developing strategies that allow them to evade or neutralize these defenses.

EXPLORING ANTARCTIC BACTERIOPHAGES

Methodological approach

The Virology area of the Microbiology group at the Argentine Antarctic Institute (IAA) conducts research on virus-host coevolution mechanisms in bacteriophage-bacteria systems, with special emphasis on the study of prophages integrated into the genome of Antarctic bacteria isolated from extreme environments (soil, continental and marine waters). Our project has the following main objectives: (1) the genomic and functional characterization of these viral elements, with particular attention to their role in the adaptive and evolutionary processes of their bacterial hosts; and (2) the evaluation of their biotechnological potential. Complementarily, through comparative genomic analyses, we investigate the molecular mechanisms involved in the acquisition of mobile genetic elements, the patterns of prophage integration, and bacterial defense systems against viral infections (Figure 1).

Lysogenic state in Bizionia argentinensis

Bizionia argentinensis JUB59, is a psychrotolerant bacterium (tolerates growth at low temperatures) isolated from the surface marine waters of Potter Cove, Carlini Base, Argentine Antarctica (Bercovich et al. 2008) (Figure 2).
This bacterium contains a prophage that parasitizes it. It was isolated and fully sequenced in Argentina as part of the White Genome Project, a public-private interinstitutional project involving the Argentine Antarctic Institute and the National Antarctic Directorate (genetic database access code: AFXZ01000000). Within this project, different proteins of unknown function were expressed and structurally characterized. Among them, the crystal structure of C24, a structural protein genetically homologous to the tail fiber of a phage, was characterized. Through induction with Mitomycin C, it was demonstrated that the C24 protein was a structural protein of an inducible prophage (Pellizza et al. 2020) (Figure 4).

Figure 2. *Geographic location where the studies were carried out, Argentine Antarctica, Potter Cove, Carlini Base.*

Bioinformatic analysis of the defense systems present in B. argentinensis allowed the identification of several innate immunity systems (Restriction and Modification, retron, pycar, and DMS). Furthermore, in silico studies identified a potential endolysin with peptidase activity, which we have named EndoBap (Endolysin of Bizionia argentinensis prophage). Functional and structural analysis of this lysis system will not only expand our basic knowledge of these mechanisms but will also open

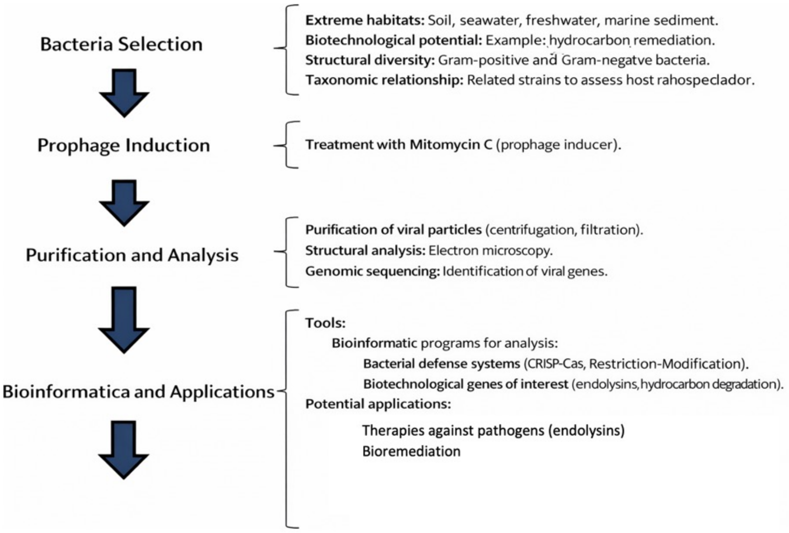

Figure 3. *Methodology of study of the virology area of the Argentine Antarctic Institute (IAA).*

Lysogenic state in Rhodococcus sp. strain ADH

Rhodococcus sp. strain ADH is a psychrotolerant hydrocarbon-degrading bacterium isolated from soil near Carlini Base, Antarctica, contaminated with petroleum-derived fuels (Ruberto et al. 2005). Induction with Mitomycin C demonstrated the presence of an inducible prophage in the bacterium. The bacterial genome was sequenced (accession number). Analysis of the defense systems present in the bacterium identified several innate immune systems (Restriction and Modification, Wadjet, and DISARM), an adaptive immune system (CRISPR-Cas type IVB), and an abortive defense system (Abi). (Figure 4).

Lysogenic state in Antarctic endophytic bacteria

The endophytic bacteria Agreia sp. CGGE2_1 and Agreia sp. CGGE2_18, which inhabit the rhizosphere of Deschampsia antarctica, an emblematic Antarctic plant, were also studied for the identification of prophapes. Their respective genomes were sequenced, and the presence of prophapes and defense systems (accession numbers) was determined. In Agreia sp. CGGE2_1, a prophage was detected after induction with Mitomycin C; while in Agreia sp. CGGE2_18, another prophage, also induced by this method, belonging to the Vilmaviridae family, was identified. Furthermore, both bacteria exhibited various defense systems. In Agreia sp. CGGE2_1, the innate immune systems DMS, retron, and Restriction and Modification were identified; while in Agreia sp. CGGE2_18 detected dXTPase, Restriction Modification and retron (Figure 6A and 6B).

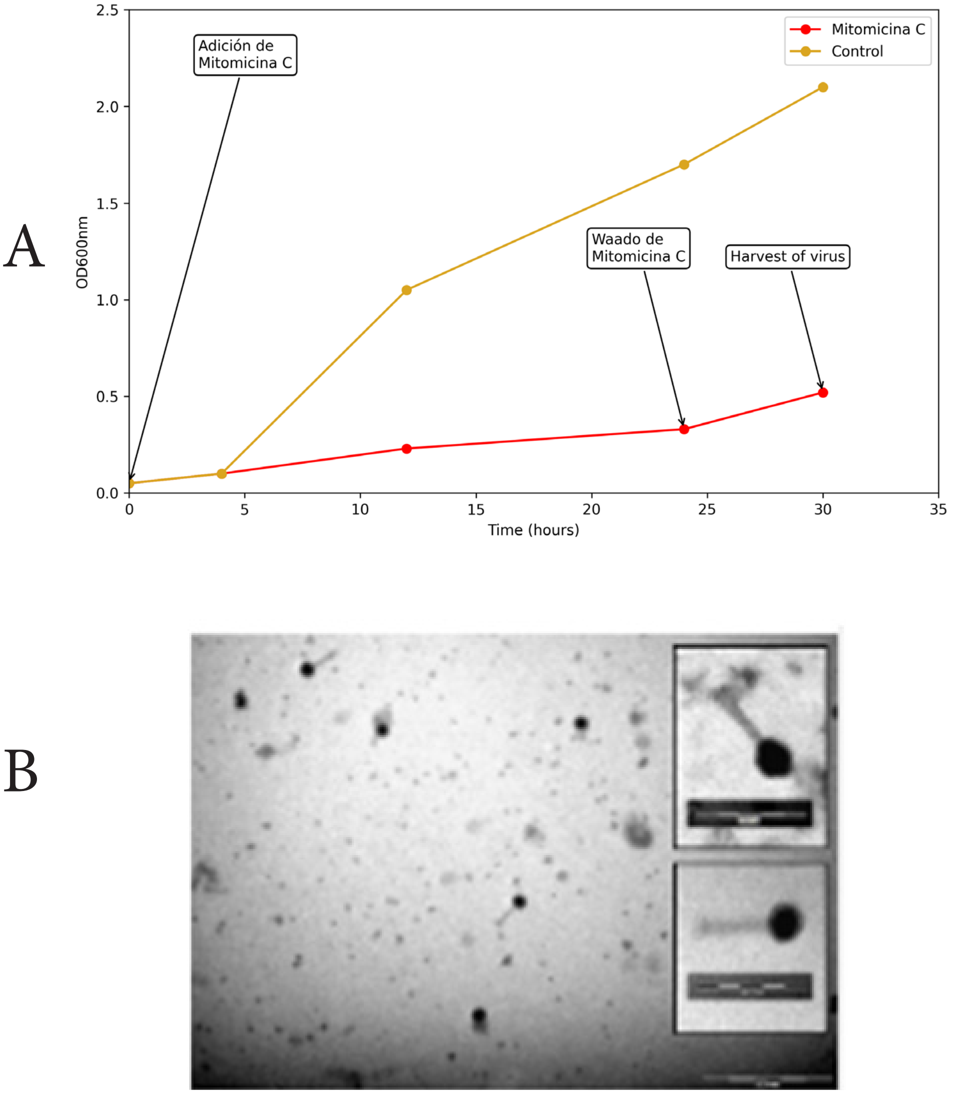

Figure 4. *A) Induction of a Bizionia argentinensis JUB59 culture. Bacterial cultures in liquid medium were performed in parallel with and without treatment with Mitomycin C. The OD600nm is a measure of light beam interference and directly measures the turbidity of the culture (turbidity is directly proportional to the number of bacteria in the culture). The decrease in culture turbidity (lower OD600nm, Figure 4A) constituted a preliminary indication of bacterial rupture by the action of released phages. B) Electron microscope image obtained by negative staining with phosphotungstic acid of the phage concentrate from the cultures treated with Mitomycin C. The electron microscope image constitutes strong evidence of prophage induction from the bacterial genome.*

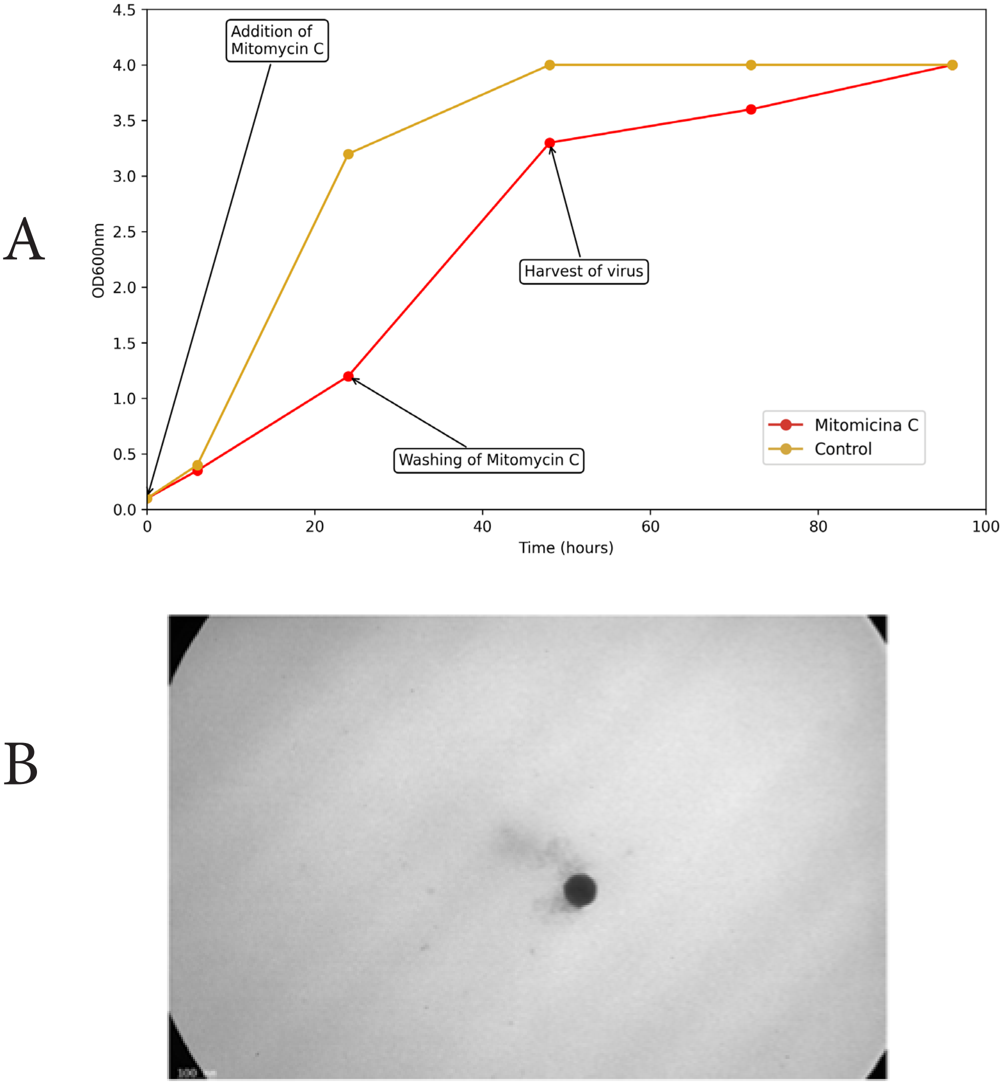

Figure 5. *A) Induction of a Rhodococcus sp. ADH strain culture. Bacterial cultures in liquid medium were performed in parallel with and without treatment with Mitomycin C. The OD600nm is a measure of light beam interference and directly measures the turbidity of the culture (turbidity is directly proportional to the number of bacteria in the culture). The decrease in culture turbidity (lower OD600nm, Figures 5A and 5B) constituted a preliminary indication of bacterial rupture by the action of released phages (compare the red curve versus the yellow curve). B) Electron microscope image obtained by negative staining with phosphotungstic acid of the phage concentrate from the cultures treated with Mitomycin C. The electron microscope image constitutes strong evidence of prophage induction from the bacterial genome.*

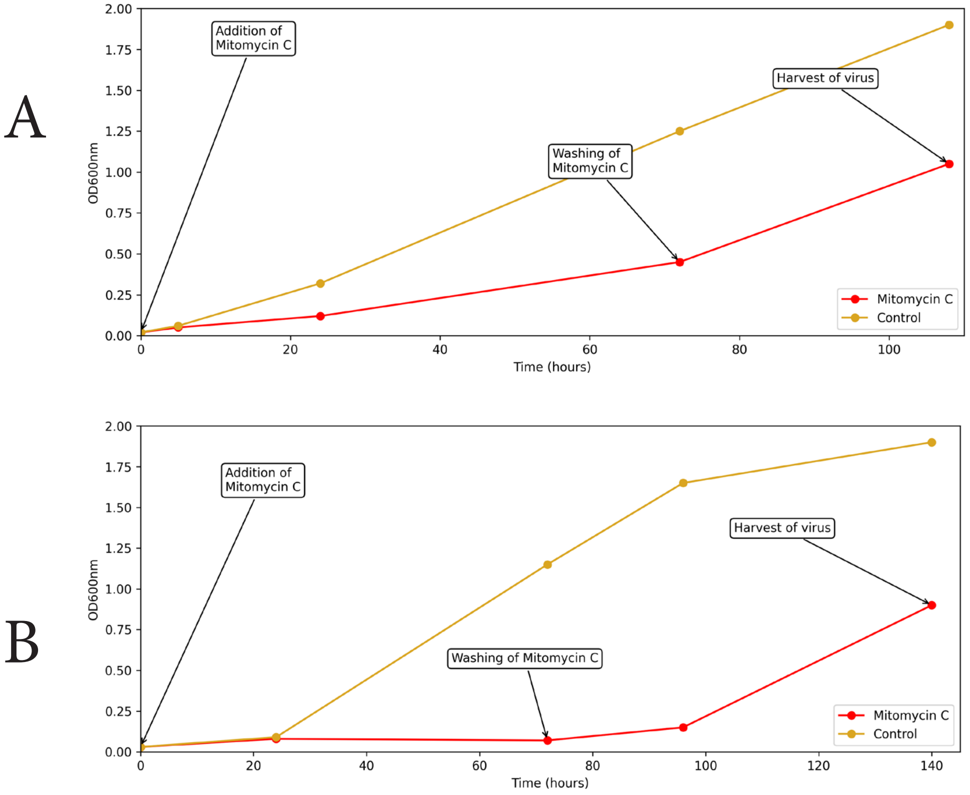

Figure 6. *A) Induction of Agreia sp. cultures CGGE2_1 B) Induction of Agreia sp. CGGE2_18. Bacterial cultures in liquid medium were performed in parallel with and without treatment with Mitomycin C. The OD600nm is a measure of light beam interference and directly measures the turbidity of the culture (turbidity is directly proportional to the number of bacteria in the culture). The decrease in culture turbidity (lower OD600nm, Figures 6A and 6B) constituted a preliminary indication of bacterial rupture by the action of released phages (compare the red curve versus the yellow curve). The electron microscope image provides strong evidence of prophage induction from the bacterial genome.*

Detailed bioinformatic analysis of the prophage genetic content revealed that approximately 25% of the genes were related to structural and viral replication functions (viral hit in the pie chart). The remaining 75% (non-viral hit) were auxiliary metabolic genes that could modulate host metabolism. This suggests that prophages can have a significant impact on bacterial cell physiology. The encoded proteins would have functions including protein binding, nucleic acid (DNA and RNA) binding, and the presence of transmembrane domains (Dom™). These transmembrane domains, when located at the amino-terminal end of the protein, constitute a molecular signal that allows secretion outside the cell (Figures 7A and 7B). These extracellular proteins encoded by the viral genome exit the lysogenic bacterium and impact plant root physiology.

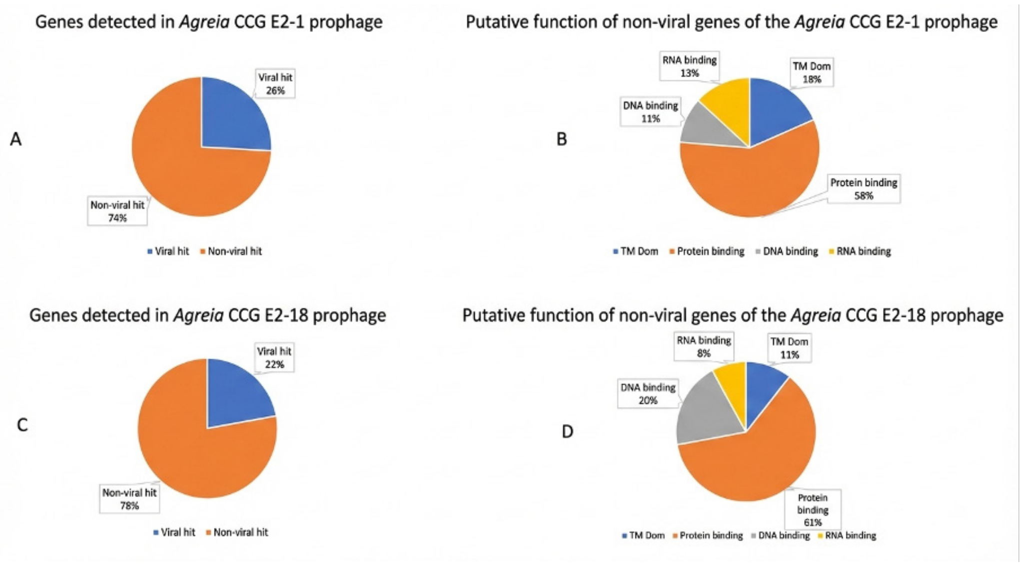

Figure 7. *A) Genes present in the prophage of Agreia sp. CGGE2_1. B) Putative function of non-viral genes present in the prophage of Agreia sp. CGGE2_1. C) Genes present in the prophage of Agreia sp. CGGE2_18. D) Putative function of non-viral genes present in the prophage of Agreia sp. CGGE2_18.*

Conclusions

The results of these studies provide preliminary evidence on the coevolution between prophages and Antarctic bacteria, their adaptation to extreme environments, and their biotechnological potential. The bacteria studied, from diverse Antarctic habitats, reveal how prophages not only play a crucial role in the evolution of their hosts but also in the diversification of their defense strategies. Analysis of the defense systems of Antarctic bacteria shows a remarkable variety of innate, acquired, and abortive immune systems, which protect them against viral invasion.

The identified genes, such as the EndoBap endolysin from B. argentinensis, represent promising biotechnological tools with specific applications in cold environments. This finding is contextualized within the growing industrial use of bacteriophage systems, where endolysins such as CF-301 (Exebacase) are used against MRSA, and formulations such as ListShield™ and SalmoFresh® are employed in the control of Listeria spp. and Salmonella spp. in the food industry. Endolysins and proteases derived from prophages have potential applications in combating pathogenic bacteria and in industrial processes, especially in sectors where low-temperature stability is crucial.

The discovery that approximately 75% of the prophage genes identified in Agreia sp. CGGE2_1 and Agreia sp. CGGE2_18 correspond to auxiliary metabolic genes suggests that these could modulate essential cellular processes in the host, impacting its physiology and adaptive capacity. The most common predicted functions, such as binding to proteins, DNA, and RNA, suggest that prophages can act as modulators of gene expression (proteins that bind to DNA and RNA), cellular metabolism (proteins that bind to other proteins), molecular transport, or cell signaling. The identification of these protein domains reinforces the hypothesis that lysogeny modulates coevolution between viruses and bacteria.

This study not only expands our understanding of the biology of prophages and their hosts in extreme environments but also highlights the value of these microorganisms as biotechnological resources. Taken together, the results not only underscore the genetic and functional biodiversity of Antarctic microorganisms but also their relevance as a model for understanding evolution in extreme environments and as a source of innovative solutions to global challenges. These include:

1) The use of Antarctic prophages as a promising alternative against the rise of antibiotic resistance—declared by the WHO as one of the greatest threats to health.

2) The development of bioremediation strategies adapted to cold ecosystems to address climate change and environmental pollution.

3) New methods of natural food preservation and more sustainable industrial processes that benefit global food security.

Finally, these findings are highly relevant within the framework of the Antarctic Treaty System. First, they corroborate Antarctica's status as a natural laboratory for cutting-edge scientific research, as established in Article II of the Antarctic Treaty. Second, they demonstrate how Antarctic studies can be translated into technological applications with global reach, in accordance with the environmental protection principles set forth in the Madrid Protocol.

AUTHOR CONTRIBUTIONS

NAN and JLL performed the experimental work and data analysis. All five authors contributed to manuscript writing and editing. NAN is the corresponding author.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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