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## Poster

## Computational analysis of a bacteriophage pangenome with multiple defense systems



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## **ABSTRACT**

**Motivation:** Antimicrobial resistance has been declared as one of the biggest threats to human health. The World Health Organization (WHO) has described a list of pathogens for which the development of new treatments is essential. One of these pathogens is *Acinetobacter baumannii*, which is classified with "Priority 1" [1]. *A. baumannii* carries different antiviral defense systems. In our group, nearly 10,000 genomes of *A. baumannii* were analyzed and in half of them, a bacteriophage, DgiS1, appeared integrated downstream of the ssrA gene. In most cases, the phage presents defense systems which seems to antagonyze with the CRISPR-Cas system from another phage [2]. For this reason, we are characterizing the DgiS1 genome, with particular focus on its defense systems and genes, which will help to shed light on the constant warfare between bacteria and phages.

**Methods:** The pangenome of DgiS1 was created using Roary Version 3.13.0 [3], with protein-coding genes previously predicted by Prokka Version 1.14.6. Proteins were functionally annotated using Sma3s v2. To identify defense systems, DefenseFinder v2.0.0 software was used [4]. In order to represent the strains features of DgiS1, heatmaps were built with R programming language. AlphaFold3 and USAlign Version 20240510 were used to analyze the structural similarity.

**Results:** 2,199 genomes of DgiS1 were analyzed and they presented an average of 93 genes. The core genome was composed of 77 cluster genes while its accessory genome had 223 cluster genes. Due to differences in its accessory genome, strains were classified in six groups, based on the presence/absence of those genes. The annotation of the genome showed that core genes were phage genes and accessory genes were mostly defense genes. Furthermore, DefenseFinder identified an antitoxin belonging to a toxin-antitoxin system in five of these groups, while in the remaining one, another type of defense system was identified in the same region. The analysis of the closest proteins to the antitoxin and its sequential and structural comparison with other reference proteins suggested the presence of a potential toxin.

**Conclusions:** DgiS1 presents a core genome composed mainly of phage-annotated genes, while its accessory genome includes a diverse range of defense systems. Strains with different types of defense systems suggest an evolutionary pressure, likely resulting from the fight that happens against other bacteriophages and to guarantee its permanence in the host bacteria.

## **REFERENCES**

- 1- Nayak, S. et al. (2025). Bacteriophage induces modifications in outer membrane protein expression and antibiotic susceptibility in Acinetobacter baumannii. Int J Biol Macromol. Vol 298, 139589. doi: 10.1016/j.ijbiomac,2025,139589
- 2- Rubio, A. et al. (2024) Biological warfare between two bacterial viruses in a defense archipelago sheds light on the spread of CRISPR-Cas systems. Cell Reports, Vol 43, Issue 12, 115085. doi: 10.1016/j.celrep.2024.115085
- 3- Andrew, J. et al. (2015). Roary: Rapid large-scale prokaryote pan genome analysis. Bioinformatics. 31(22):3691-3693. doi: 10.1093/bioinformatics/btv421
- 4- Tesson, F. et al. (2022). Systematic and quantitative view of the antiviral arsenal of prokaryotes. Nature Communication. 13:2561. doi: 10.1038/s41467-022-30269-9