Analysis of the association between bacteriophages and specific strains of *Pseudomonas aeruginosa*

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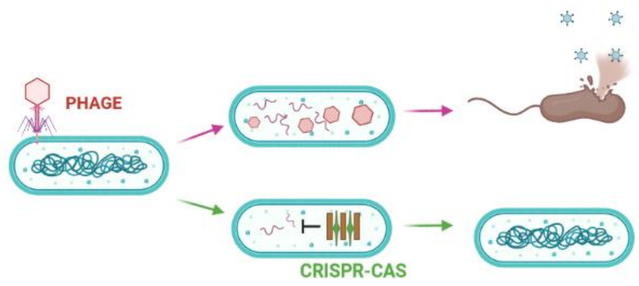
Bacterial infections has always been considered one of the most crucial challenge in the clinical field until the discovery and use of penicillin. However. the indiscriminate use of antibiotics has led to the emergence of multi-resistant bacterial strains. This leads us to the use of alternative therapies to antibiotics, such as phage therapy, which uses viruses to deal with bacteria. For the correct use of this therapy, it is completely necessary to know which phages infect each bacterium and what their features are. In addition, it is also useful to know what are the defense systems used for bacteria to deal with these viruses. By analyzing 7836 genomes from *Pseudomonas aeruginosa* bacteria, we have identified all prophages integrated in their genomes and we have related them with different features of the bacterial strains, such as the geographical location, the isolation source or CRISPR-Cas systems. These results show a co-ocurrence of some phages and CRISPR-Cas systems, while the rest of strain features do not correlate. This allows the elaboration of a catalog of phages positively and negatively associated with the different types of CRISPR-Cas systems, which is potentially useful for the application of phage therapy.

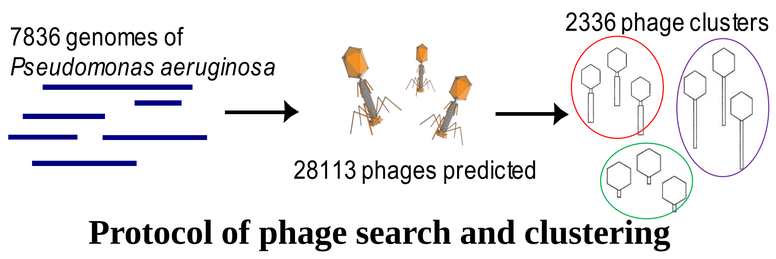
Phages **infect** bacteria to replicate themselves using bacterial machinery, This **virulent process** ends in the lysis of bacterial membrane and the liberation of the phage copies. In some cases, phages can integrate into the bacterial genome as **temperate phages** or **prophages**, that are those sequences which we predicted from genomes.

To deal with these exogenous agents, bacteria have developed different **defense systems**, such as **CRISPR-Cas systems**, which can recognize phages in a specific way and remove them.



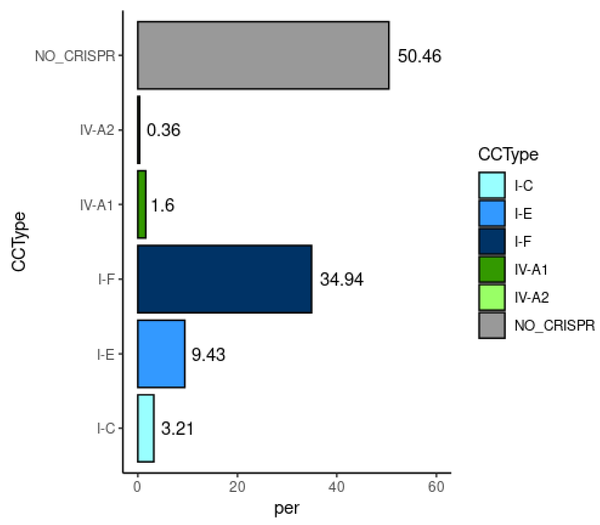
Infective and defensive process of bacteriophages

Phage sequences were searched for and classified by taxonomy (**Phigaro**) in all genomes of *Pseudomonas aeruginosa*. In order to eliminate redundancy between sequences, phages were clustered by similarity (**MeShClust**).



Protocol of phage search and clustering

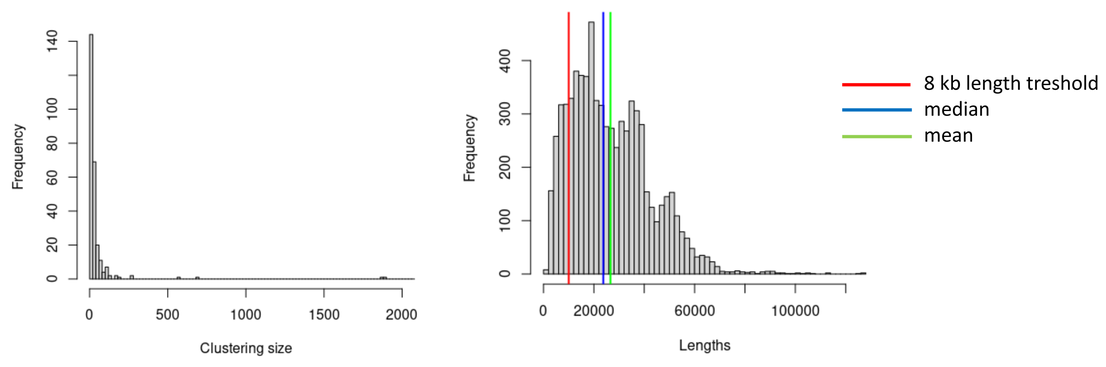
Also, CRISPR-Cas systems were searched for in *Pseudomonas aeruginosa* genomes (**CRISPRCasTyper**). Spacers of these defense systems were identified (**CRISPRCasFinder**).



CRISPR-Cas types in Pseudomonas aeruginosa

Barplots and statistical analysis were performed through R scripts using the **ggplot2** and **dhyper** library, respectively.

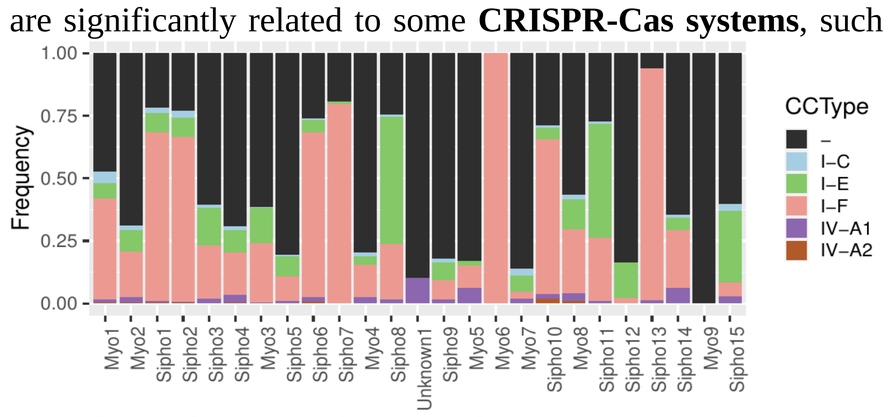
We were able to determine the wide **diversity of phages** in *Pseudomonas aeruginosa* thanks to a deep clustering process and the use of multiple strain metadata. We considered as a complete phage any sequence larger than 8 kb.



Clustering size distribution (left) and distribution of phage lengths (right)

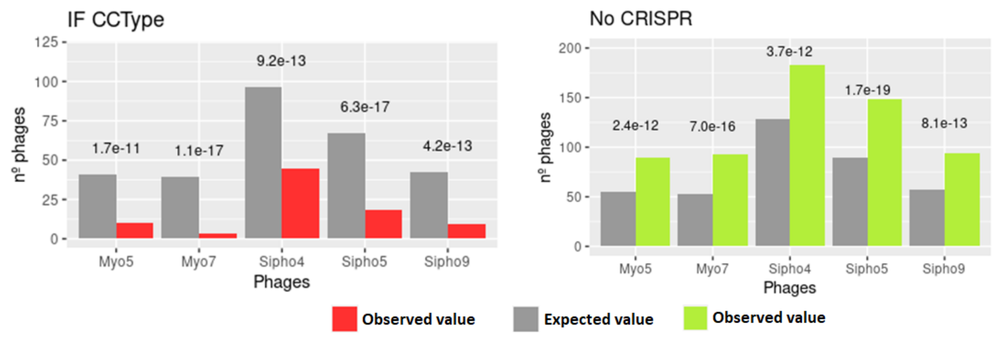
Some of these features, such as geographic location or isolation source, did not correlate with phages.

According to the defense systems, most of phages are in strains without CRISPR-Cas systems, which is to be expected since bacteria use these systems to face them. However, some phages are significantly related to some **CRISPR-Cas systems**, such as **I-F** or **I-E** types.



Correlation between phages and CRISPR-Cas systems

We searched for spacers against phages related to strains without CRISPR-Cas systems to know **what type of CRISPR-Cas system faces these phages**. Phages which are **significantly increased in strains no CRISPR** were **significantly decreased** in strains with CRISPR-Cas systems, particularly with **type I-F systems**. This type of CRISPR-Cas system has **spacers** against these phages.



Decreased and increased number of phages in I-F CRISPR strains and no CRISPR strains

Knowing which bacteriophages can infect a certain bacterial species, as well as how bacteria defend themselves against these viruses, is essential for a better understanding of bacterial biology and for developing alternative approaches in the battle against bacteria, such as phage therapy.

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