Phage Lysins as a Promising Alternative Class of Antibiotics: A Metagenomics-Driven High-Throughput Platform for the Discovery of Novel Lysins

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BACKGROUND

Antibiotic-resistant bacteria are projected to kill 10 million people by 2050. Moreover, there has been a lack of introduction of new antibiotic classes for over five decades Consequently, this resulted in an increasingly reduced number of therapeutic options. Hence, there is an urgent need for the development of new discovery platforms to identify novel antibacterials and **replenish the antibiotic portfolio**.

Lysins, or bacteriophage-encoded peptidoglycan hydrolases, represent a promising and alternative class of antibiotics. They are usually highly specific at the species level, resulting in a narrow spectrum of activity. Yet, more novel lysin candidates must be discovered and engineered to feed the (pre)clinical pipeline.

In this regard, my research focuses on two approaches to find novel lysins: **functional and sequence-based metagenomic methodologies**. Their main idea rests upon the premise that the **largely unexplored metagenome** derived from uncultivable bacteriophages represents an essentially infinite reservoir of potentially potent lysins.

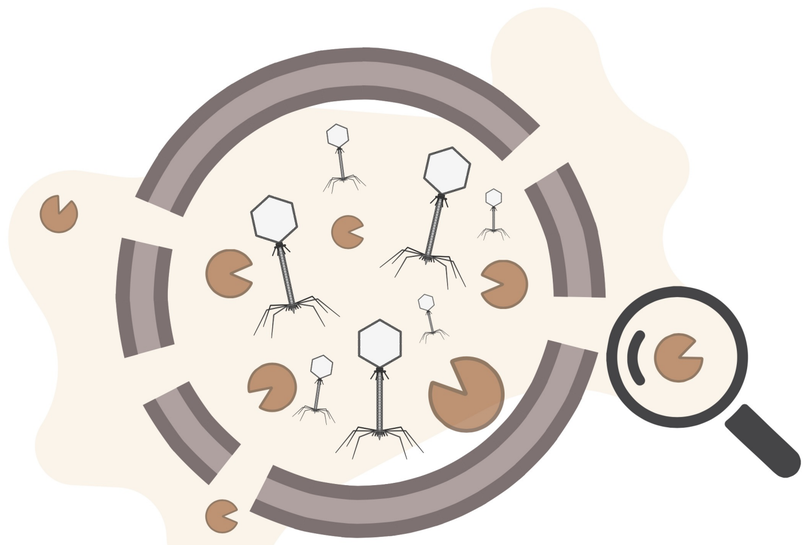

Figure created with BioRender

FUNCTIONAL METAGENOMICS

On the one hand, an approach can be elucidated based on functional metagenomics. This methodology relies on constructing and screening metagenomic libraries that are created with **phage DNA extracted from environmental samples**. This way, novel lysins can be detected based on their **activity**. *Figure 1* gives an overview of the different steps of the workflow towards the creation of (meta)genomic libraries.

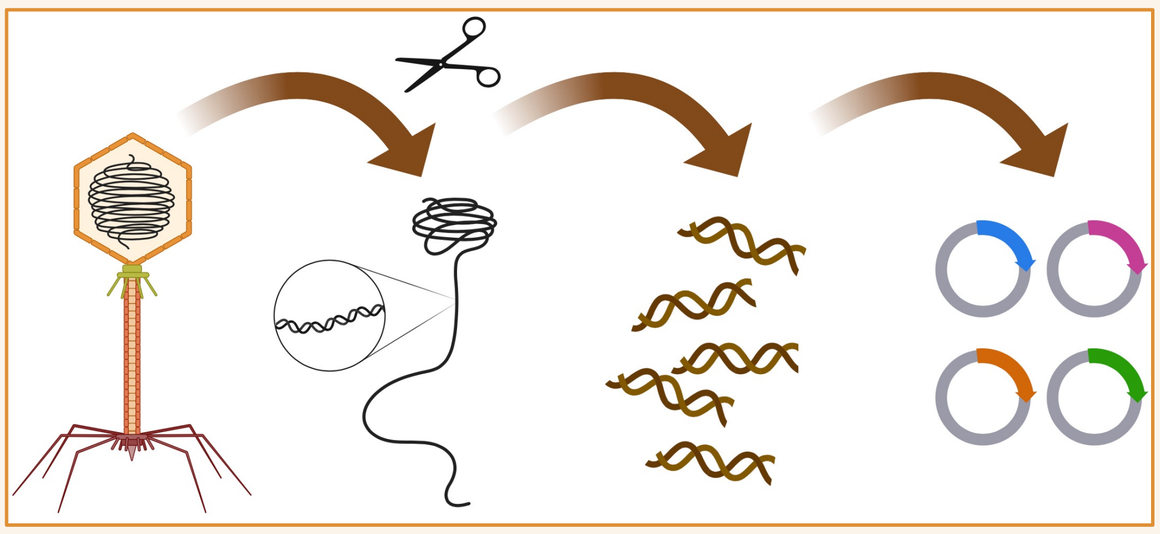

Figure 1. *Workflow to create (meta)genomic libraries.* *(Figure created with BioRender)*

A (meta)genomic library can be constructed with following subsequent steps:

- Collecting bacteriophages from environmental samples
- Extracting the phage DNA
- Enzymatic fragmentation
- Adaptor ligation (to enable controlled amplification and cloning)
- Amplification of the DNA fragments
- Relevant size selection
- Cloning into an expression vector (and transformation of suitable host)

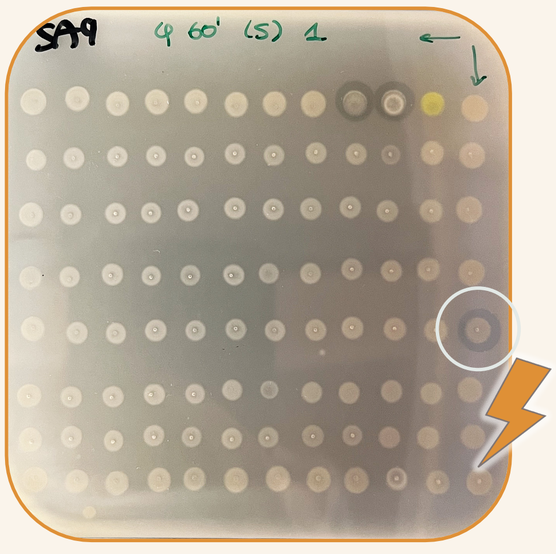

Figure 2. *Halo-assay with a genomic library.*

When the libraries are constructed, we screen them by performing **halo assays**. In these assays, the expressed fragments are screened for muralytic activity.

The fragments that contain an active lysin will cause degradation of the peptidoglycan of the target bacteria included in the agar around the colony. This results in a halo and gives an easy visual detection of activity.

Figure 2 gives an example of a halo-assay in which a library is screened, started from a pure phage culture (positive control). This clone contained the endolysin gene on its plasmid.

SEQUENCE-BASED METAGENOMICS

On the other hand, the discovery of novel lysins can also be addressed **bioinformatically**. This implies searching for metagenomic viral datasets, curating the data, selecting potentially interesting DNA sequences based on **characteristics** (architecture, host, environment, …) and lastly, analysing the selected proteins on activity.

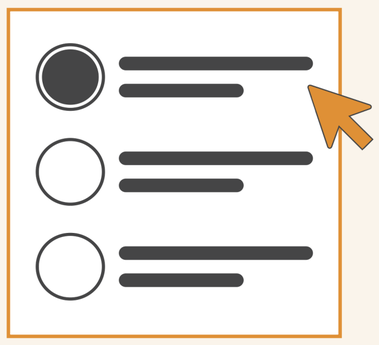

Figure: Noun Project

In my research, I chose a curated dataset[2] containing putative endolysins that were predicted from a database of uncultured phage genomes, and I selected nine interesting predicted endolysins.

The next steps to analyse these potential lysins are:

- Cloning the gene into an expression vector
- Express proteins
- Perform activity assays, *e.g.,* turbidity reduction assay (TRA)

Two lysins have already been tested for muralytic activity against (outer membrane permeabilised) Gram-negative *Pseudomonas aeruginosa* (PAO1) with a **turbidity reduction assay**. The results are illustrated in *Figure 3*.

Endolysin **RVLD_177** clearly degrades the peptidoglycan of the bacterial target, whereas endolysin **RVLD_1085** shows no cleavage since it has the same turbidity decrease as the **negative control**.

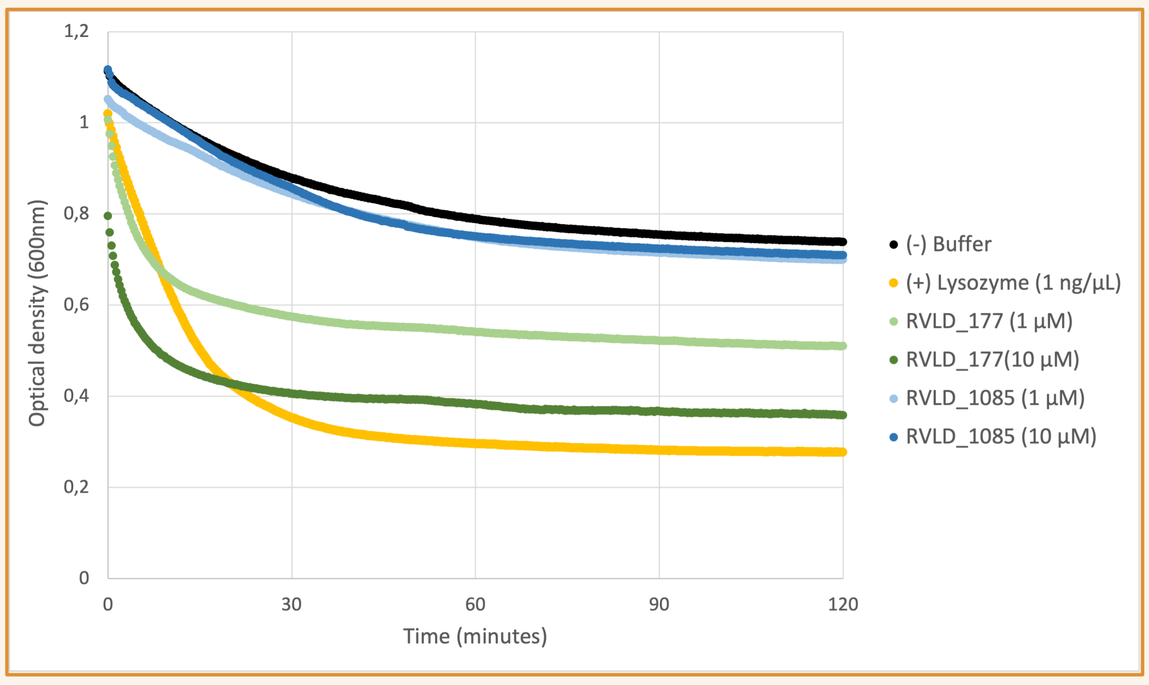

Figure 3. *Results turbidity reduction assay. Target bacteria: PAO1. Negative control: protein buffer. Positive control: Henn egg white lysozyme. Lysins: RVLD_177 and RVLD_1085.*

The endolysin RVLD_177 was predicted with an unknown enzymatically active domain, after blasting against the NCBI database. So, a highly new active enzymatically domain has been discovered, which now requires in-depth analysis.

CONCLUSION & FUTURE PERSPECTIVES

Both approaches are very promising solutions to replenish the number of lysin candidates for their further evaluation as potential antibacterials. **Functional metagenomics** allows to identify radically new lysin sequences showing muralytic activity in unsequenced metagenomic samples but is labour intensive. **Sequence-based metagenomics** is a simpler method useful to find new lysin sequences but relies on (low) sequence similarity with known phage lysin sequences, thus preventing to identify completely novel lysin sequences. Both methods can be complementary and broaden the portfolio of promising phage lysins.

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[2] Fernández-Ruiz, I., Coutinho, F. H., & Rodriguez-Valera, F. (2018). Thousands of novel endolysins discovered in uncultured phage genomes. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2018.01033