

# Phage discovery: isolation and characterization of seventeen novel anti-*Staphylococcus aureus* bacteriophages

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**PHAGEinLYON**

## INTRODUCTION

The rapid inexorable spread of antibiotic resistance is one of the critical challenges in health care for the coming decade. **Phage therapy** uses viruses known as bacteriophages, that specifically infect bacteria, and is a promising alternative to antibiotic therapy. In this context, the **PHAGEinLYON consortium** aims to develop an academic production of therapeutic phages, targeting different multi-resistant bacteria such as *Staphylococcus aureus* and complying with expectancies of drug agencies. The first step of this project consists in the set up of a **large collection of bacteriophages and assessment of their therapeutic potential**.

**Aim:** In this study, we evaluated the activity of seventeen recently isolated phages against a collection of well-characterized clinical strains representative of the major *S. aureus* clones.

## METHODS

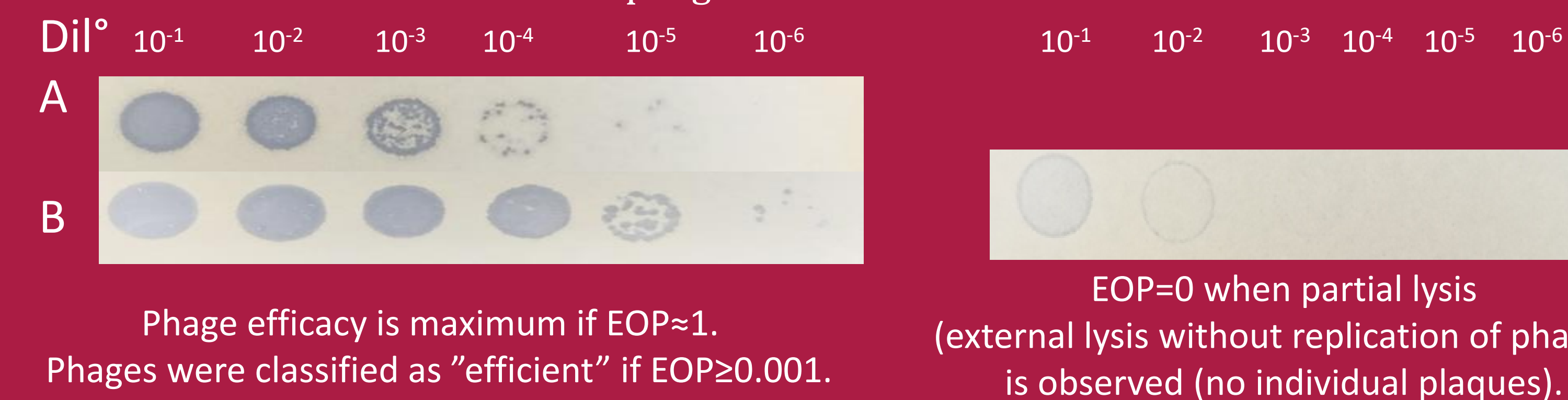
### Bacterial strains

The panel included 30 genetically-characterized strains (DNA array StaphyType, Alere Technologies, Germany) susceptible (MSSA, n=17) or resistant (MRSA, n=13) to methicillin.

### Phages

**Seventeen *Myoviridae* phages** belonging to two genera (*Kayvirus*, n=13; *Silviavirus*, n=3) isolated from wastewater samples were included in this study. We used the **spot test assay** to assess their activity spectrum with the determination of the **Efficiency Of Plating** ratio:

$$EOP = \frac{A = \text{phage titer on a test strain}}{B = \text{phage titer on a reference strain}}$$



## RESULTS

### *Silviavirus* phages were more efficient than *Kayvirus* phages on *S. aureus* isolates :

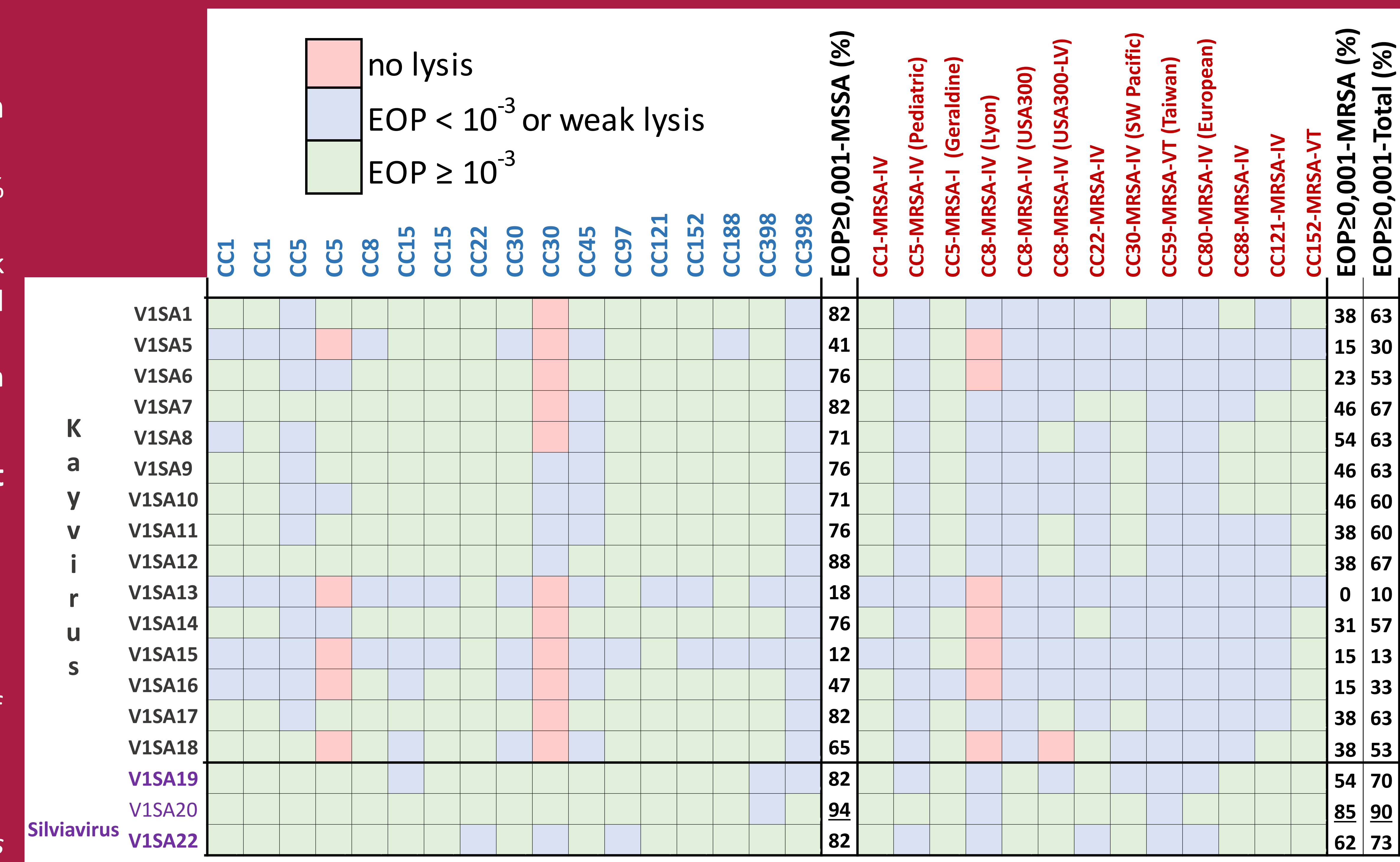
- We observed an EOP ≥ 10<sup>-3</sup> for 70 to 90% or 10 to 67% of strains respectively
- For other strains, we observed an EOP < 10<sup>-3</sup> or a weak lysis for almost all phages. For three strains, we did not observe any lysis for several *Kayvirus* phages.
- Phage **V1SA20** had the widest activity spectrum (EOP ≥ 10<sup>-3</sup> for 90% of strains)

### Phages were significantly more efficient against MSSA than MRSA strains :

- Median percentage of strains with EOP ≥ 10<sup>-3</sup> : 76% vs 38% for MSSA and MRSA strains respectively (p < 0.001, Mann-Whitney test)
- Silviavirus* were more active than *Kayvirus* phages on MRSA strains : EOP ≥ 10<sup>-3</sup> for 50 to 85% or 0 to 54% of MRSA strains respectively

### Complementarity of activity spectra :

We observed EOP ≥ 10<sup>-3</sup> with at least one *Kayvirus* and one *Silviavirus* phages for 77% of strains allowing combinations of phages of different genera to possibly prevent emergence of phage resistance for these strains.



**Figure 1:** Evaluation of the activity spectrum of seventeen novel *Myoviridae* phages (*Kayvirus*, n=14; *Silviavirus*, n=3) against a panel of 30 *Staphylococcus aureus* clinical strains. MSSA strains are indicated in blue, MRSA in red

## CONCLUSIONS

We report the identification and preliminary characterization of the activity spectrum of **seventeen novel phages belonging to two different genera with broad activity** against *S. aureus*. Further evaluation of the activity of the subset of the most interesting phages will be performed on a larger panel of strains with complementary techniques and assessment of synergistic effects between *Kayvirus* and *Silviavirus* phages.

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