

Characterization and efficacy evaluation in the *Galleria mellonella* model of four novel bacteriophages targeting *Aeromonas salmonicida*



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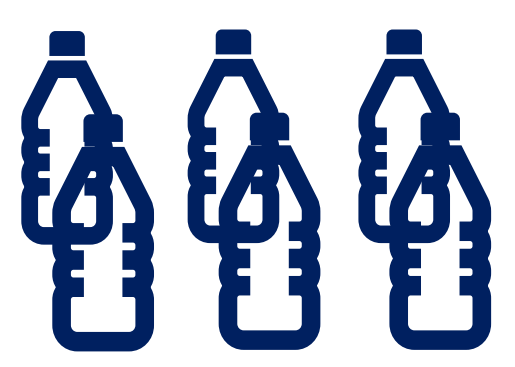
BACKGROUND The Gram-negative bacterium *Aeromonas salmonicida* is a primary fish pathogen that causes **furunculosis** in **salmonids** as well as septicemia in a variety of fish. In view of the major consequences of furunculosis in salmonid productions worldwide and the frightening tendency of *A. salmonicida* to exhibit antimicrobial (multi-)resistances, **phage therapy** could represent a leading alternative to treat this infection in aquaculture. This study aimed to **1 isolate bacteriophages** targeting *A. salmonicida*, **2 characterize them *in vitro***, and **3 evaluate their ability to improve the survival** of infected *Galleria mellonella* larvae. To further **4 investigate phage-bacteria interactions** in this model, phage and bacterial concentrations were monitored throughout the experiment.



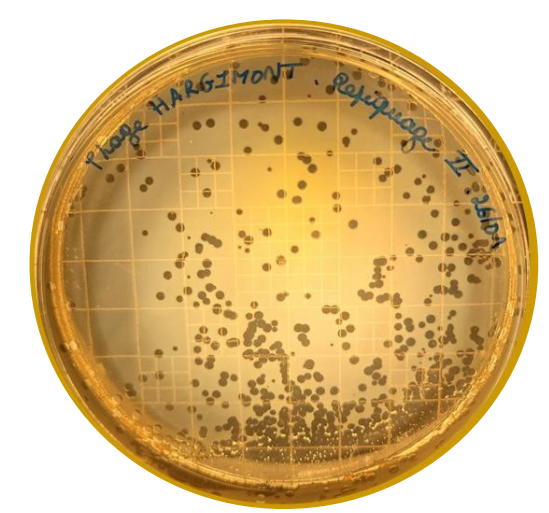
External ulcerative lesions on a rainbow trout *Oncorhynchus mykiss* caused by *A. salmonicida* (from Menanteau-Ledouble et al., 2016)

METHODS

1 PHAGE ISOLATION

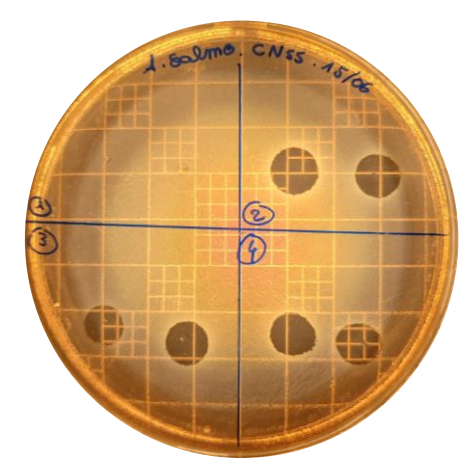


Water sample collection



Enrichment method

2 IN VITRO CHARACTERIZATION



Host spectrum

T° & pH stability

Sequencing & analysis

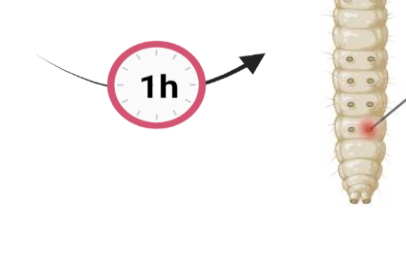
Transmission electron microscopy

3 SURVIVAL IN GALLERIA MELLONELLA LARVAE

Grouping



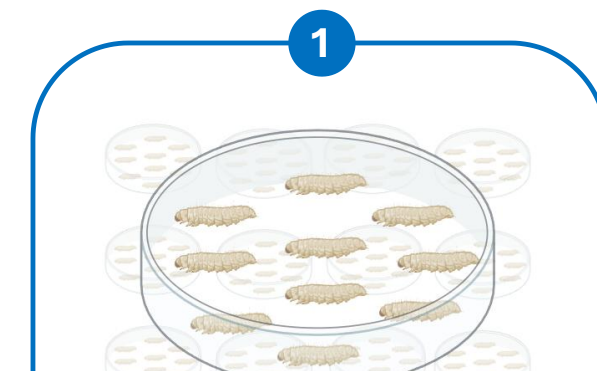
Inoculations



Larval survival rate evaluation (4 days)

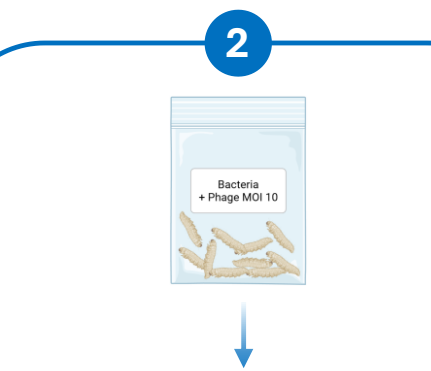
4 PHAGE & BACTERIA TITRATIONS IN GALLERIA MELLONELLA LARVAE

Grouping & inoculations



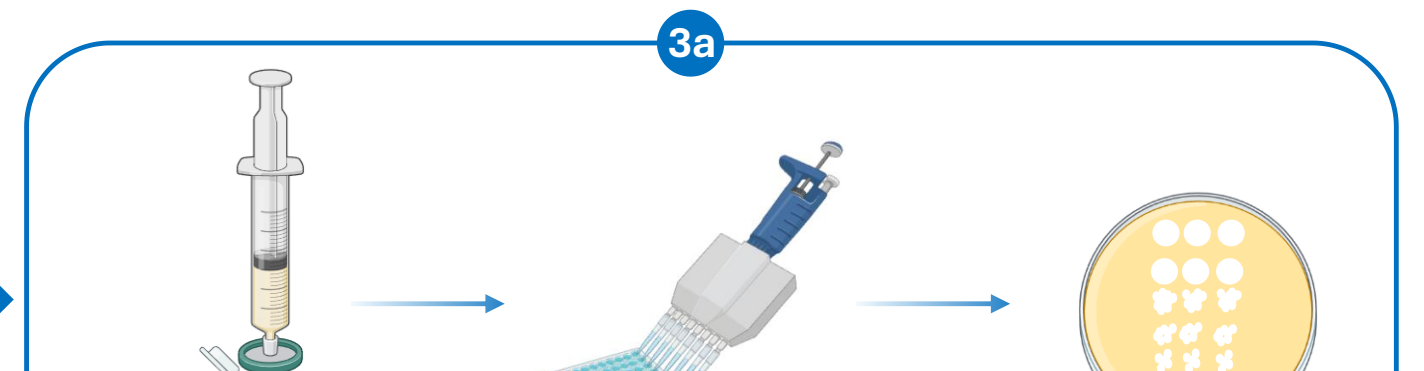
Bacteria + Phage MOI 10
Bacteria + Phage MOI 100
Bacteria + PBS
PBS + Phage MOI 100
PBS + PBS

Larvae mixing

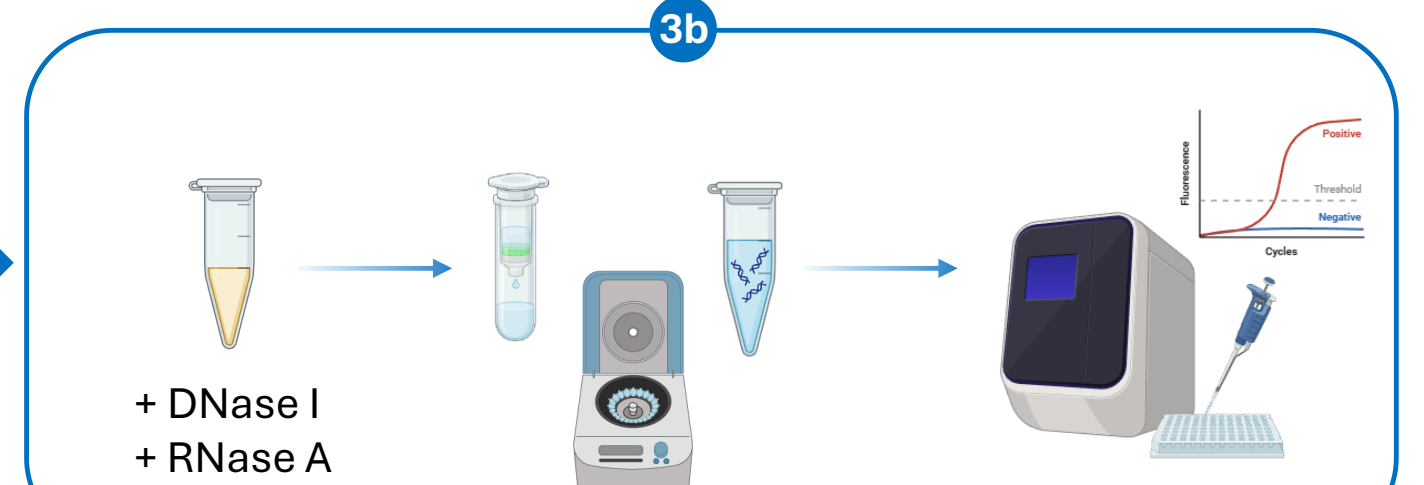


PBS + 200 µl larva mixture

Phage titrations

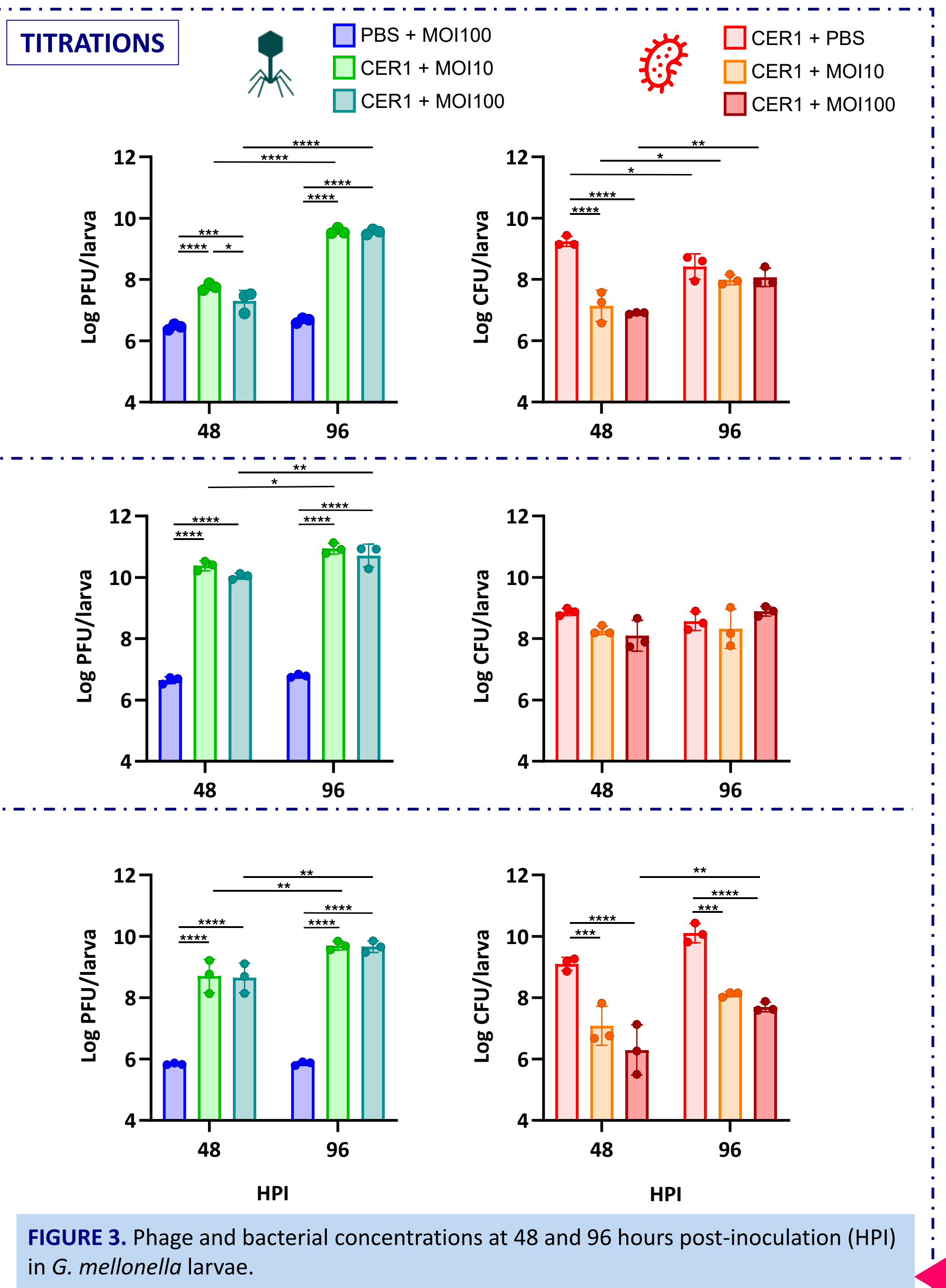
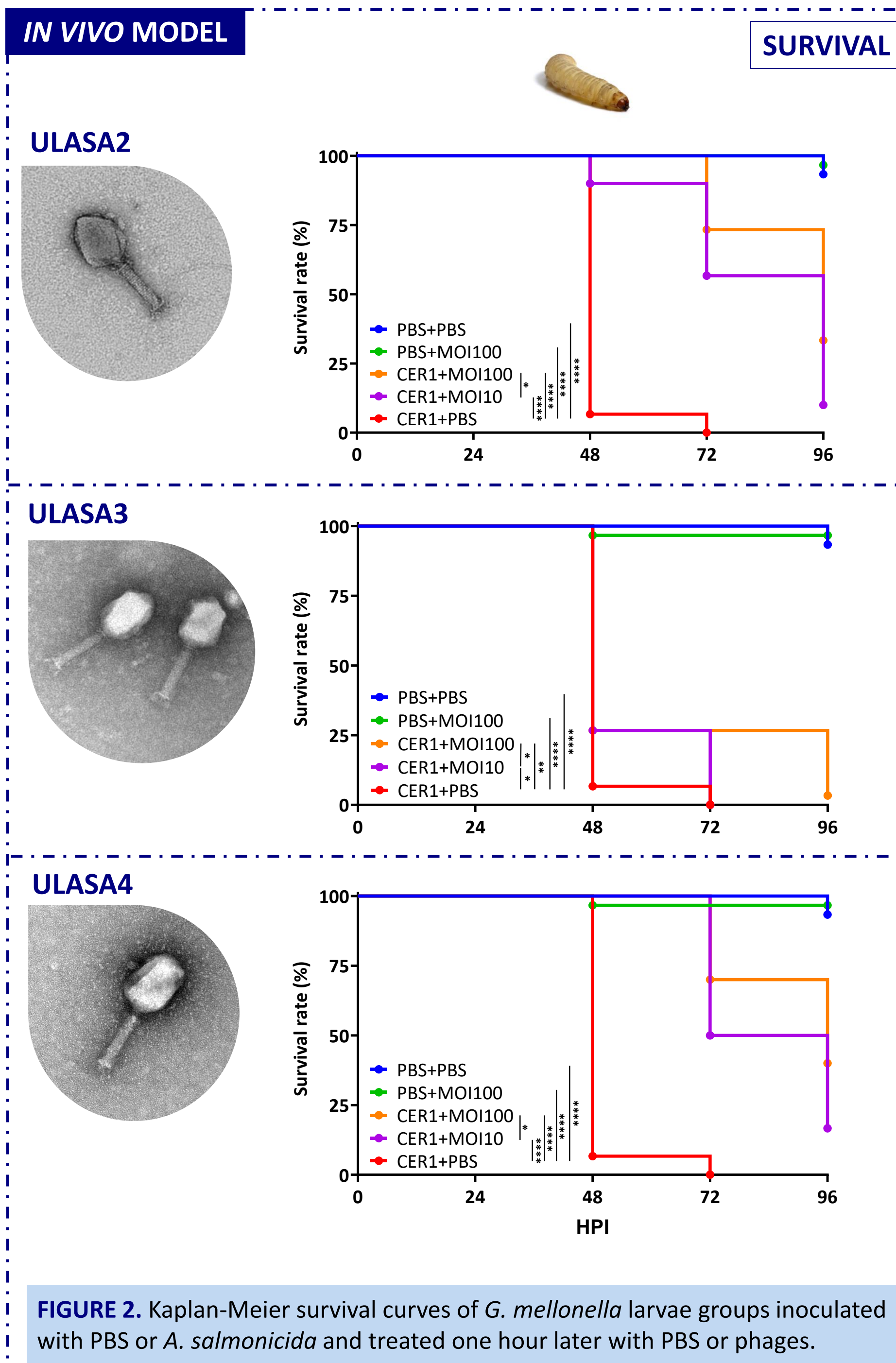
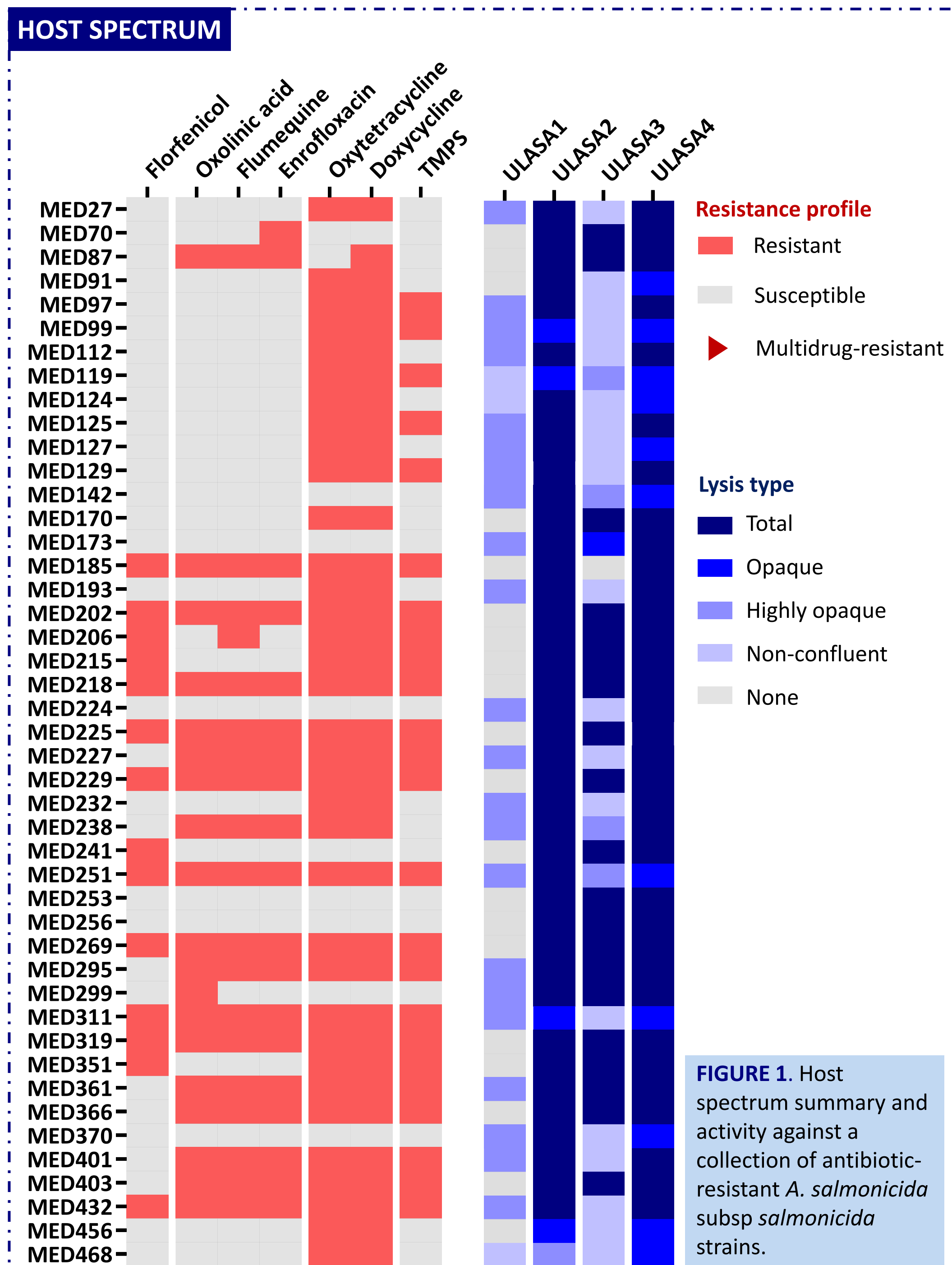


Bacterial titrations



DAY 0 DAY 1 DAY 2 DAY 3 DAY 4

RESULTS Four novel phages presenting a **myovirus morphotype** were isolated from fish farms and aquatic environments in Belgium and France. Genomic analyses revealed that the three lytic phages vB_AsaM_ULASA2 (170,823 bp), vB_AsaM_ULASA3 (164,381 bp), and vB_AsaM_ULASA4 (171,205 bp) belong to the **Straboviridae** family, whereas no known family could be assigned to the phage vB_AsaM_ULASA1 (47,813 bp). Three of these phages were **active against other *A. salmonicida***, including strains of the pathogenic *salmonicida* subspecies displaying a multidrug-resistant profile (**FIGURE 1**), and showed a high biochemical tolerance. Single administrations of the phages ULASA2, ULASA3, and ULASA4 **improved the survival of *G. mellonella* larvae** previously infected with *A. salmonicida* (**FIGURE 2**). The three phages **successfully replicated in the model**, however only the ULASA2 and ULASA4 phages significantly **reduced bacterial concentrations** compared with untreated groups (**FIGURE 3**).



CONCLUSIONS In this study, **four phages targeting *A. salmonicida*** were isolated (vB_AsaM_ULASA1, vB_AsaM_ULASA2, vB_AsaM_ULASA3, and vB_AsaM_ULASA4). Three of these phages were **active against other strains of *A. salmonicida*** and **improved the survival of infected *G. mellonella* larvae**, even if it did not result in a complete elimination of the bacteria. Taken together, these findings highlight the **potential of phage therapy** for the management of furunculosis, contributing to the **reduction of antibiotic use** and limiting the development of antimicrobial resistance in aquaculture.

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