

Isolation, *in vitro* characterization and efficacy assessment in *Galleria mellonella* larvae of four bacteriophages targeting *Aeromonas salmonicida*

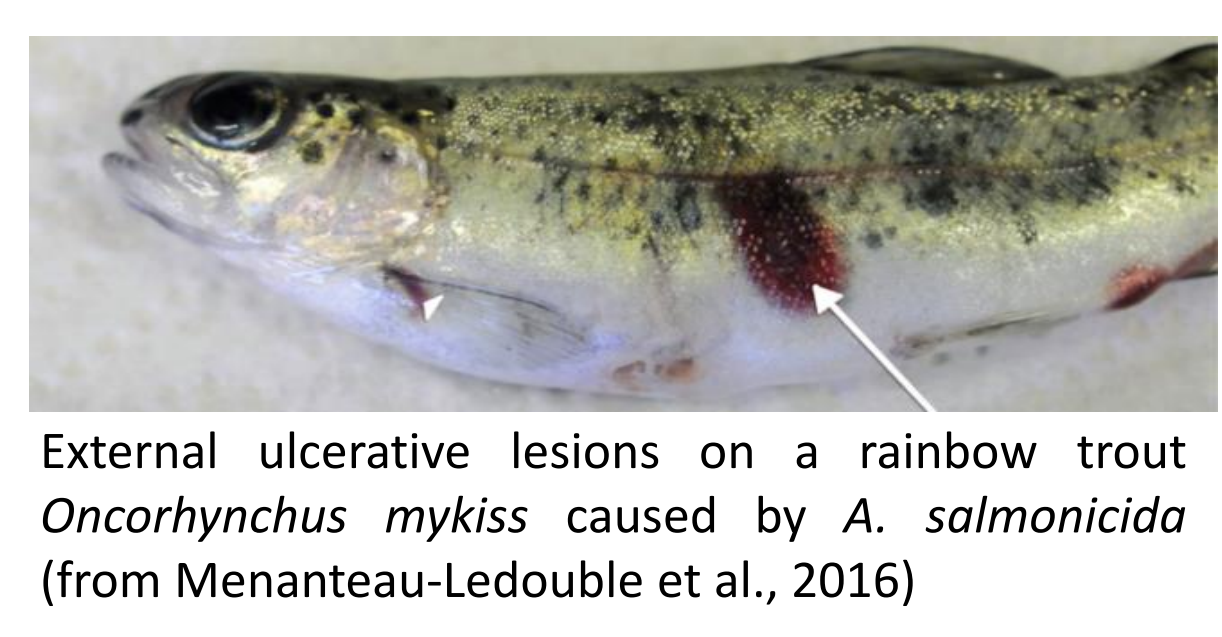


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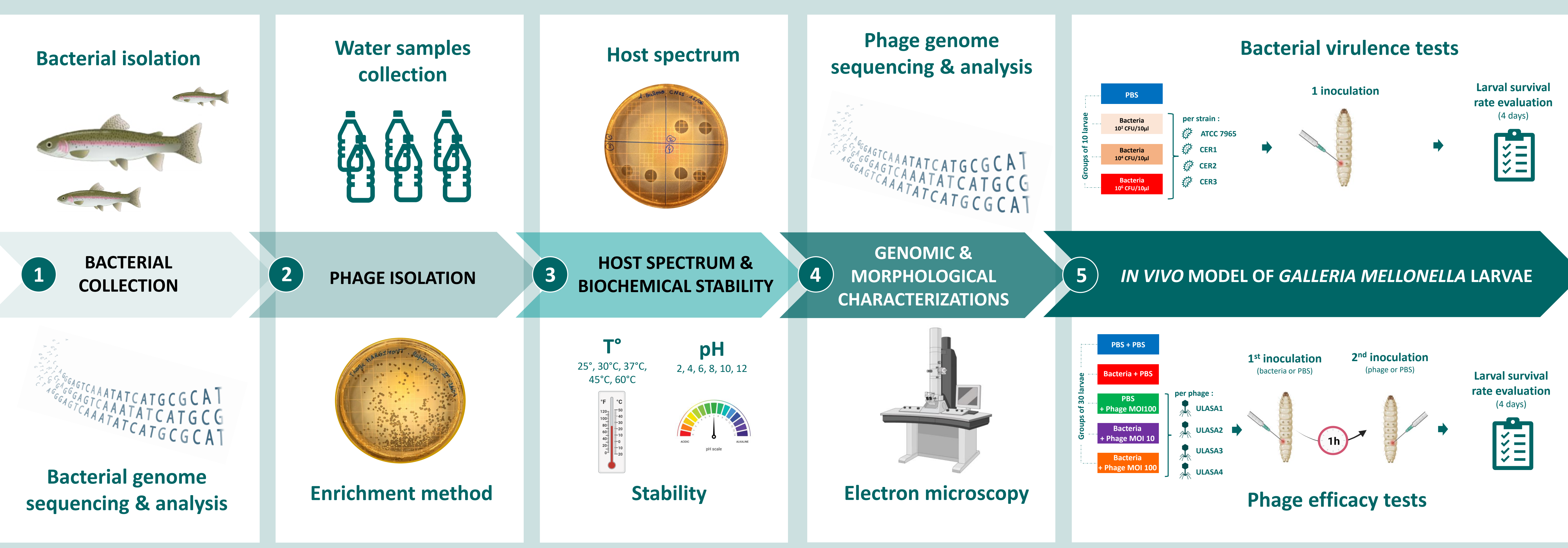
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INTRODUCTION

The Gram-negative bacteria *Aeromonas (A.) 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. The aims of this study were to **1** create a **collection of *A. salmonicida* strains**, **2** **isolate phages** targeting these strains, **3** determine their **host spectrum** and **biochemical stability**, **4** characterize their **genome** and **morphology**, and finally **5** assess their **potential for phage therapy** in the preliminary *in vivo* model of *Galleria (G.) mellonella* larvae.

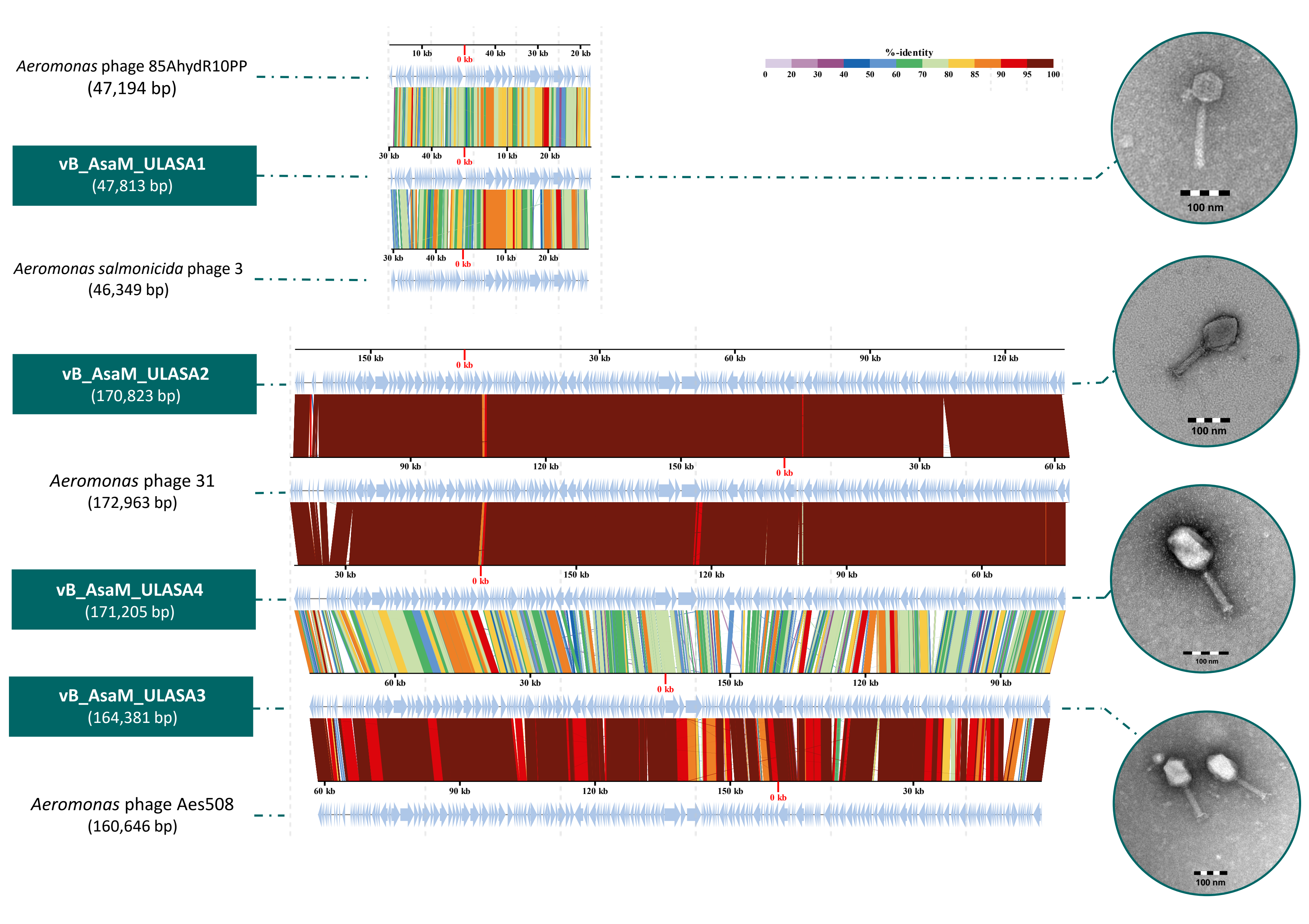


MATERIAL & METHODS

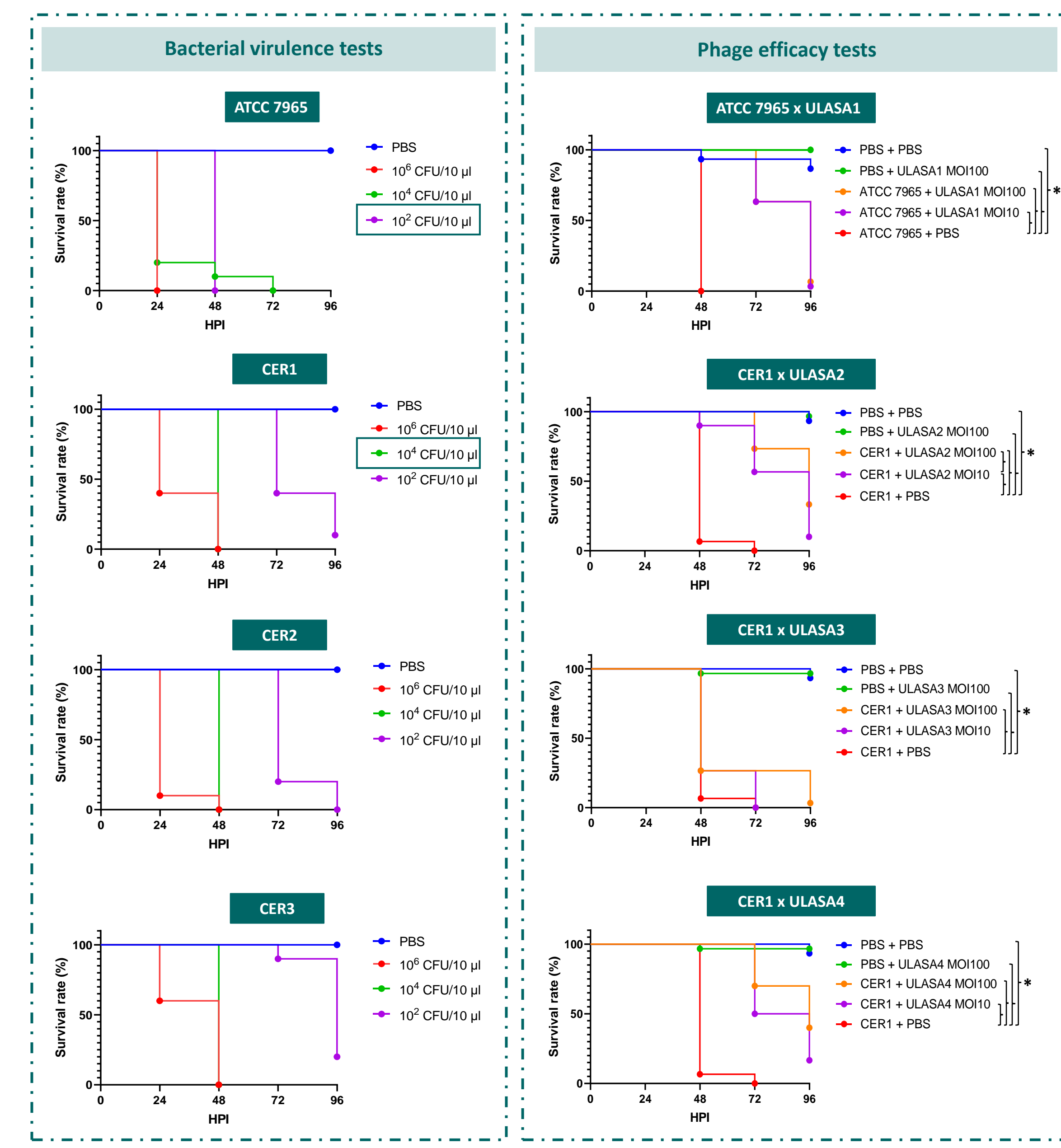


RESULTS

From a total of 157 isolation experiments, **four new phages** active against *A. salmonicida*, named vB_AsaM_ULASA1 (47,813bp), vB_AsaM_ULASA2 (170,823bp), vB_AsaM_ULASA3 (164,381bp) and vB_AsaM_ULASA4 (171,205bp), were isolated from water samples collected in fish farms and natural aquatic environments. Phages ULASA2, 3 and 4 were **active against other *A. salmonicida* strains** and showed a **high resistance to temperatures and pH** tested while the phage ULASA1 showed a more restricted host spectrum and less biochemical tolerance. Genomic analysis showed that phages ULASA2, 3 and 4 belong to the **Straboviridae family** but no family has already been attributed to the phage ULASA1. All four presented a **myovirus morphotype**.



Four strains of *A. salmonicida* were tested for virulence on *Galleria mellonella* larvae and two of them, named ATCC 7965 and CER1, were selected for further experiments. Inoculation doses were determined as 10^2 CFU/ $10 \mu\text{l}$ and 10^4 CFU/ $10 \mu\text{l}$, respectively. Four-day efficacy experiments in this infection model showed that phages **ULASA1, 2 and 4** were responsible for a **significant extension in the larval survival time** at the **two treatment doses tested** (MOI 10 and 100) while ULASA3 only showed a significant effect at MOI 100.



CONCLUSION

In this study, **4 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 showed a **high biochemical tolerance**. They also **prolonged the survival time of *G. mellonella* larvae** previously infected with *A. salmonicida*. In light of these results, these newly isolated phages could represent **potential new candidates** for the development of anti-furunculosis phage treatments in aquaculture.



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