

# Alphagos *Vibrio*, a tailored phage cocktail reduces *Vibrio parahaemolyticus* infection in Indonesian shrimp hatcheries

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## Introduction

*Vibrio parahaemolyticus* infection is a major threat to **whiteleg shrimp (*Litopenaeus vannamei*) aquaculture**, causing significant shrimp mortality and leading to considerable economic losses for hatcheries and farms. Bacteriophages provide a promising antibiotic alternatives due to their high efficacy and specificity towards target bacteria. **phagos** and **Vaksindo** Satwa Nusantara develop bacteriophage products for aquaculture. This experiment aims at determining the efficacy of bacteriophage to eliminate *Vibrio parahaemolyticus* bacteria in the maintenance environment of vannamei shrimp.

## Objective

Show the robustness of **Alphagos** *Vibrio* phage cocktail by treating naturally *V. parahaemolyticus*-infected shrimp larvae sampled from an Indonesian hatchery with a phage cocktail designed to target *V. parahaemolyticus* strains isolated from other hatcheries in Indonesia.

## Cocktail design

Designed to target 13 representative strains (but not tested on the strains infecting the shrimp larvae at the moment of the trial)  
→ isolated from 5 **Indonesian shrimp hatcheries**, from 3 islands, across more than 3000 km.

**Prediction *in silico*** of the best phage cocktails to test from **phagos** phage collection

Prediction further confirmed *in vitro* on the 13 representative strains

→ selection of a **5-phages cocktail**

## Trial design

Shrimp origin

Trial conditions

Shrimp hatchery

Post-larvae 10 confirmed *V. para pos*

transferred to

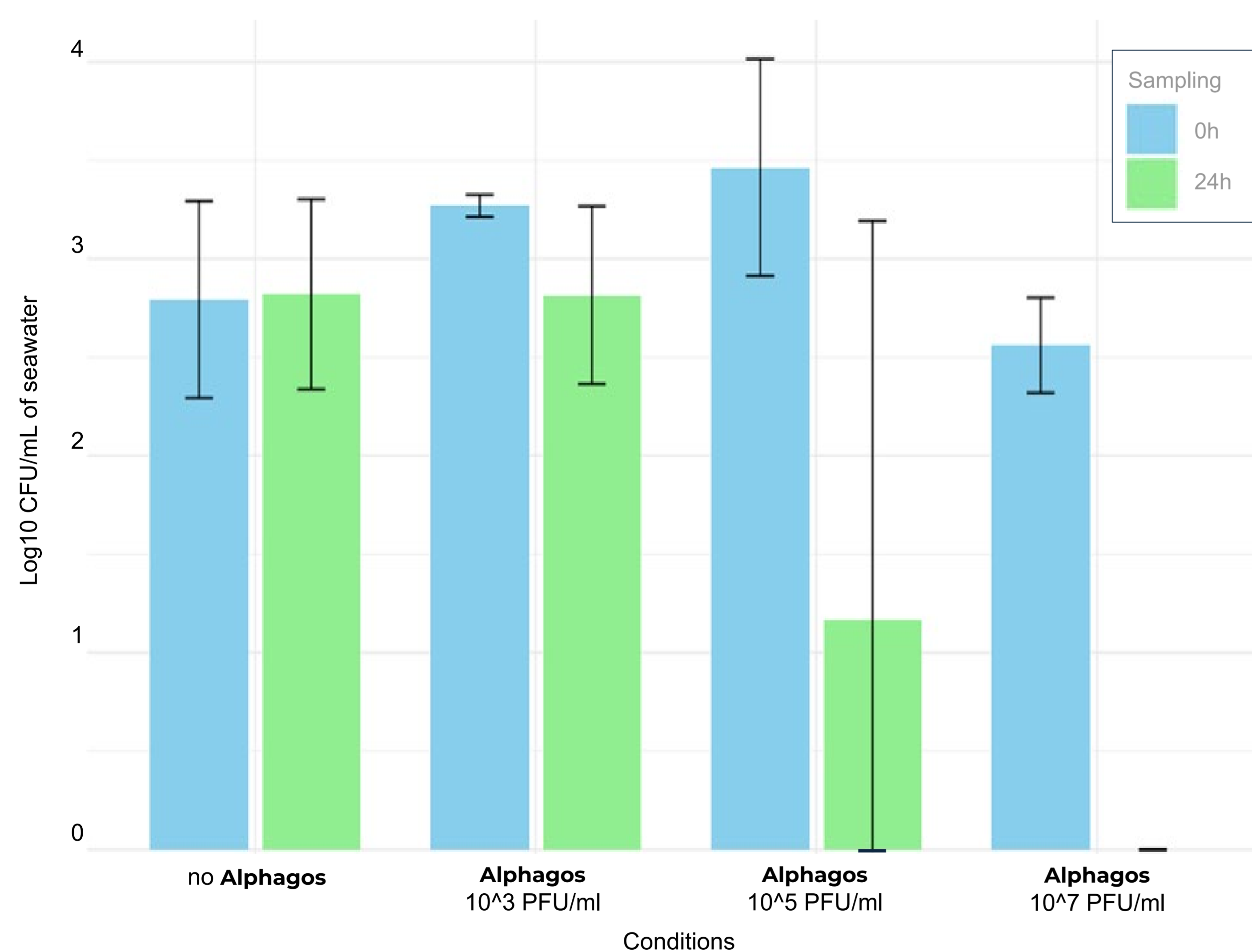
Wet lab

Alphagos trial on naturally infected PL10

| Condition | Alphagos <i>Vibrio</i> |
|-----------|------------------------|
| A         | positive control       |
| B         | 10 <sup>3</sup> PFU/mL |
| C         | 10 <sup>5</sup> PFU/mL |
| D         | 10 <sup>7</sup> PFU/mL |

- No experimental infection as PL10 naturally infected
- 3 biological replicates/condition
- Total *Vibrio* count on Chromagar Vibrio and TCBS
- *V. parahaemolyticus* count on Chromagar Vibrio

## Results



**Figure 1 - Mean *Vibrio parahaemolyticus* CFU counts represented in log scale at time 0h and 24h of the trial.** Bars represent the mean CFU counts/replicates (x3) and the error bars represent the standard deviation. CFU counts were done on seawater sampled from the trays at time 0h corresponding to the beginning of the trial and after a 24h incubation with/without phage treatment. In the positive control, there is no *V. parahaemolyticus* load difference between time 0h and 24h. When administered at 10<sup>3</sup> PFU/ml, **Alphagos** *Vibrio* does not induce a significant *V. parahaemolyticus* loads reduction in 24h while higher doses, at final concentrations of 10<sup>5</sup> and 10<sup>7</sup> PFU/mL of seawater, led to a **significant reduction** (2 replicates/3 were *V. para* negative) and **total elimination** respectively. **Alphagos** *Vibrio* had no effect on the total *Vibrio* count (data not shown).

## Key takeaways

- **Alphagos** *Vibrio* eliminates *V. parahaemolyticus* from naturally contaminated seawater.
- **Alphagos** *Vibrio* has no effect on the non *V. parahaemolyticus* *Vibrio* species.
- **Alphagos** *Vibrio* is efficient against a large spectrum of *V. parahaemolyticus* strains isolated in diverse shrimp hatchery hot spots in Indonesia, making it a great product to decontaminate stocks before the farming phase and to reduce the risk of *V. parahaemolyticus*-caused production losses.