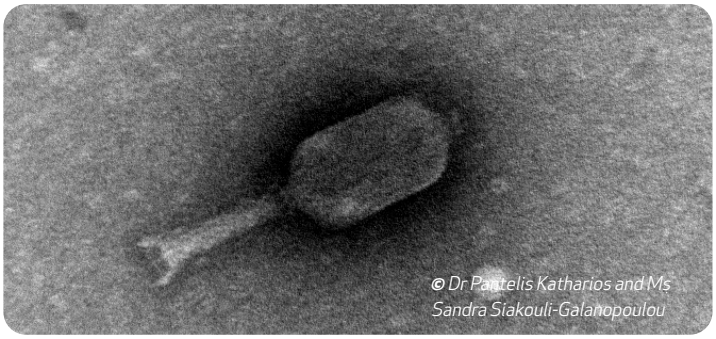
ADMINISTRATION OF LYTIC BACTERIOPHAGES TO SELECTIVELY DECREASE *VIBRIO* SPP. PATHOGENS IN AQUACULTURE HATCHERY SYSTEMS



SUMMARY

Phage therapy is the use of bacterial viruses, known as bacteriophages or phages, to selectively tackle potentially pathogenic bacteria prevalent in fragile systems such as aquaculture hatcheries. The use of biocontrol applications such as phage therapy is a promising area, and interest in the various practical applications, such as improved food and feed safety, has been gaining momentum. This research investigated the use of five bacteriophages together (two of which were previously characterised and three which were novel) to target the bacterial pathogens *Vibrio harveyi* and *Vibrio alginolyticus* in the aquaculture live feed, *Artemia salina,* as an alternative to antibiotics. The results showed that the administration of the bacteriophages had the potential to selectively decrease the populations of these *Vibrio* pathogens in the live feed.

KNOWLEDGE NEED

Bacterial diseases in aquaculture pose a serious problem for both the sustainability of production and the health and welfare of the fish. Vibriosis is one of the most common bacterial diseases in marine aquaculture hatcheries, typically caused by *Vibrio* bacteria entering larval rearing water through live feeds such as *Artemia* and rotifers. Traditionally, bacterial diseases such as vibriosis are treated with antibiotics, but overuse has led to concerns around antimicrobial resistance, environmental pollution and consumer health. Vaccines can also be used against vibriosis in later stages of fish growth, but this is not an effective strategy for larval stages. Alternative strategies to control bacterial infections are urgently needed and bacteriophages have shown potential as an effective method for biologically controlling potential outbreaks of *Vibrio* spp. in aquaculture systems. Research is in its early stages, and more information needs to be gathered, including efficacy, costs and efficiency.

UNDERLYING SCIENCE

Vibrio harveyi and *Vibrio alginolyticus* are prevalent in *Artemia salina* live feed cultures, and can use these as vehicles to infiltrate the hatchery systems, causing high mortality rates in the cultured species. Researchers isolated and characterized 5 lytic bacteriophages: St2 and Grn1 (isolated against *V. alginolyticus*), which were previously sequenced and characterized and VH2_DHL, DSM19623_DHL and V1_DHL (isolated against *V. harveyi* and *V. alginolyticus*), which have recently been isolated and sequenced. The efficacy of the phage cocktail consisting of the 5 lytic bacteriophages was first assessed *in vitro* against presumptive *Vibrio* colonies extracted from the *Artemia salina* live feeds. The phage cocktail was then deployed *in vivo* to the *Artemia salina* live feeds to selectively decrease the populations of *Vibrio harveyi* and *Vibrio alginolyticus*.

POTENTIAL IMPACT

- Healthier aquaculture environments due to selective targeting of potentially pathogenic bacteria while leaving the natural microbiota intact and not leaving any wastes in the ecosystem.
- A sustainable strategy to fight bacterial diseases and tackle antimicrobial resistance due to the level of specificity and the environmental origin of the viruses.
- Improved welfare of cultured fish.
- Improved understanding of the role of bacteriophages in tackling pathogenic bacteria.
- Supporting precaution before treatment in aquaculture health management.

EATiP - Strategic Research and Innovation Agenda (SRIA) Thematic Area 7- Aquatic Animal Health and Welfare; Goal 4. To see the full list and descriptions of the thematic areas and goals, please visit: **eatip.eu/?page_id=46**

RESULTS

- The phage cocktail was able to inhibit *in vitro* presumptive vibrios in *Artemia salina* live feeds.
- The administration of lytic bacteriophages in *Artemia salina* live feeds selectively decreased the native populations of the opportunistic pathogens *Vibrio harveyi* and *V. alginolyticus*.
- Smaller volumes of live feed cultures led to better preliminary results because the increased multiplicity of infection seems to disinfect the live feed more efficiently.

END-USERS & POTENTIAL APPLICATIONS

END-USER 1: Producers of lytic bacteriophages
APPLICATION: This research supports a demand for increased production of lytic bacteriophages to better manage pathogenic bacteria in aquaculture systems.

END-USER 2: Marine hatchery farmers and managers
APPLICATION: Aquaculture farmers could more sustainably manage pathogenic bacteria by using bacteriophages instead of antibiotics, tackling both waste in the environment and antimicrobial resistance.

END-USER 3: Producers of live aquaculture feeds
APPLICATION: As an alternative to antibiotic administration, producers of live aquaculture feeds could use bacteriophages to selectively remove bacterial pathogens in their feed.

END-USER 4: Marine microbiologists and fish pathologists
APPLICATION: The results highlight the potential use of lytic bacteriophages in treating pathogenic bacteria in an aquaculture environment.

END-USER 5: Consumers
APPLICATION: Consumption of safe fish products that have been produced in an antibiotic-free environment.

END-USER 6: Policy makers
APPLICATION: The results highlight that bacteriophage therapy is a potent alternative to antibiotics. Policy makers could use results such as these to define a clear legislatory path that will allow for the commercialization of phage therapy products while ensuring their safe use in the EU.

STATUS

Technology Readiness Level (TRL) 5 – technology validated in relevant environment (industrially relevant environment in the case of key enabling technologies).

- The methodology needs to be more finely tuned (e.g. the quantity of bacteriophages), work is ongoing in this area.
- Further research is needed to ensure that up-scaled production of bacteriophages is purified from negative by-products (e.g. toxins) that may originate from the bacteriophage lysis events that occur during proliferation.
- The proliferation protocols for the bacteriophages need to be improved to maximise phage concentrations prior to phage therapy treatment.
- Trials are required to formulate a fully efficient phage cocktail (including research on its components in case of competitive interactions among the phages), and also to expand phage therapy trials against other bacterial targets.
- The output owner has participated in the setup of a new company (aquatic-biologicals.com), which will carry out more work on this topic at a commercial level.

AT A GLANCE

TITLE: Administration of lytic bacteriophages to selectively decrease *Vibrio* spp. pathogens in aquaculture hatchery systems

KNOWLEDGE TYPE: Exploitable scientific result

WHERE TO FIND IT: Contact details below

STATUS: The research is continuing

TNA FACILITY USED: Hellenic Center for Marine Research (HCMR), Greece

CONTACT DETAILS: Panagiotis Kalatzis, University of Copenhagen, Denmark; panos.kalatzis@bio.ku.dk

PATENTS OR OTHER IPR EXPLOITATIONS: N/A

Designed and developed by AquaTT

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 652831. This output reflects the views only of the author(s), and the European Commission cannot be held responsible for any use which may be made of the information contained therein.