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## Phage therapy to inactivate pathogenic bacteria in aquaculture

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Worldwide, fish and bivalve aquaculture increased its importance in order to compensate the reduction of natural populations. One of the major sources of financial loss for fish farming industry is the development of infections by pathogenic bacteria, namely multidrug-resistant ones. This problem is most prominent in the early stages of fish development, and it is hard to address with traditional antibiotic or vaccination. Filter feeder bivalve molluscs, due to their filtration feeding, growth and collection in contaminated seabed and, the fact of frequently being consumed raw or light cook, bivalves, are responsible for transmission of numerous food-borne diseases to consumers. Presently, the prevention of outbreaks transmission relies on the depuration process, which despite the fact of being useful in the elimination of several microorganisms is inefficient in the elimination of others, including pathogenic bacteria. Therefore, new environmentally-friendly strategies to control pathogenic bacteria in aquaculture are urgently needed. Phage therapy appears to represent a useful and flexible tool for the inactivation of bacterial pathogens in aquaculture. The aim of the present study was to test the efficacy of phage therapy applied during the production of juvenile flounder fish and during cockle depuration. In the production of juvenile flounder fish, phage therapy was used to inactivate *Aeromonas salmonicida*, which is the causative agent of furunculosis, a systemic disease characterized by high mortality and morbidity in aquaculture. The results showed that AS-A phage inhibited the growth of the *A. salmonicida*, causing a decrease in bacterial abundance of  $\approx 3$  CFU mL<sup>-1</sup> log after 6 h of treatment. After 72 h, the mortality of juvenile fish was higher (34%) in the fish exposed to *A. salmonicida* but not treated with phages than in the infected and treated groups (0%). The addition of AS-A phage to aquaculture water have not showed a detectable impact on the structure of the natural bacterial community. However, the bacteriome of the fish was significantly affected by the addition of the AS-A phage, but the differences were lower when the phage was added in the presence of the host bacteria. In the bivalves decontamination, two phages (phT4A and ECA2) of *Escherichia coli* and two phages (phSE-2 and phSE-5) of *Salmonella enterica* serovar Typhimurium (*salmonella typhimurium*) were used during depuration of natural and artificially contaminated cockles (*Cerastoderma edule*) in depuration systems with (mimicking industrial depuration conditions) and without recirculating water. The results showed that, for both bacteria, approximately 2 log were inactivated in artificially contaminated cockles and approximately 0.6 – 0.9 log for *E. coli* and *salmonella typhimurium* in naturally contaminated ones. It was also found that the use of phages during depuration decreased bacterial concentration faster than the use of depuration alone, as to achieve the same bacterial concentration decrease; two more hours were needed if no phage was used. Development of bacterial resistance to phages was observed ( $\sim 1 \times 10^{-4}$  -  $1 \times 10^{-5}$  CFU g<sup>-1</sup>), but mutant of *E. coli* and *salmonella typhimurium* grew much slower and their colonies were smaller than the susceptible ones.

### Biography

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