

APPROACHES TOWARDS BIO-ENCAPSULATION OF ORAL VACCINES FOR EARLY STAGES OF AQUACULTURED FISH



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Introduction

By developing a targeted vaccination strategy of early stages of fish, important fish diseases can be prevented in the European aquaculture industry

We aimed to develop a protocol for oral vaccination of fish larvae via live prey as a part of EU project TargetFish. This work was done in collaboration with Ansgar Stratmann (W42), Simona Bartkova (DTU-VET) and Louise von Gersdorff Jørgensen (KU-SUND) who have provided recombinant Green Fluorescent Protein (GFP) expressing *Pichia pastoris*, *Aeromonas salmonicida* and *Yersinia ruckeri* respectively.

Aeromonas salmonicida is a bacterium responsible for the disease furunculosis and *Yersinia ruckeri* is a bacterium responsible for enteric red mouth disease. *Pichia pastoris* is a yeast widely used as a vector for biotherapeutic purposes using recombinant DNA-techniques.

It was assumed that the prey will accumulate inactivated antigens in their digestive tract, the live prey being then fed to the fish larvae as biocapsules, acting as vaccines for fish in the early stages, when it is otherwise difficult to vaccinate fish. Tests were made to see if marine copepods *Tigriopus californicus*, marine rotifers *Brachionus plicatilis* and freshwater *Daphnia magna* will ingest *P. pastoris* yeast, *A. salmonicida* and *Y. ruckeri* bacterins that had GFP integrated in them.

Setup

Different concentrations of yeast and bacteria with GFP were fed to all stages of *Daphnia*, copepods and rotifers.

The zooplankton was tested on whitefish larvae (*Coregonus lavaretus*), flounder larvae (*Platichthys flesus*) and trout larvae (*Oncorhynchus mykiss*). Functional testing of the immune response is in progress.

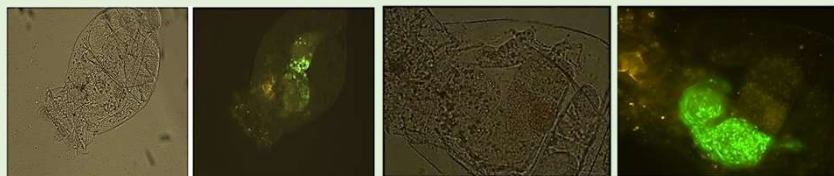


Fig. 1. *Brachionus plicatilis* rotifer photographed without fluorescent light (left) and with fluorescent light (right). The rotifer has *Aeromonas salmonicida* GFP bacteria in its digestive tract.

Fig. 2. *Brachionus plicatilis* rotifer photographed without fluorescent light (left) and with fluorescent light (right). The rotifer has *Yersinia ruckeri* GFP bacteria in its digestive tract.

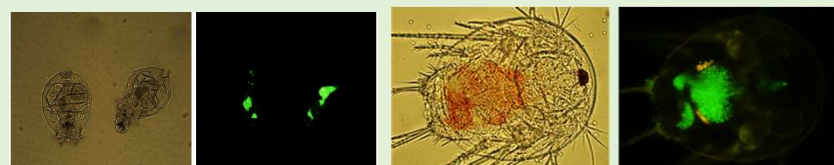


Fig. 3. *Brachionus plicatilis* rotifers photographed without fluorescent light (left) and with fluorescent light (right). The rotifers have *Pichia pastoris* GFP yeast cells in their digestive tract.

Fig. 4. *Tigriopus californicus* nauplii photographed without fluorescent light (left) and with fluorescent light (right). The nauplii has *Pichia pastoris* GFP yeast cells in its digestive tract.

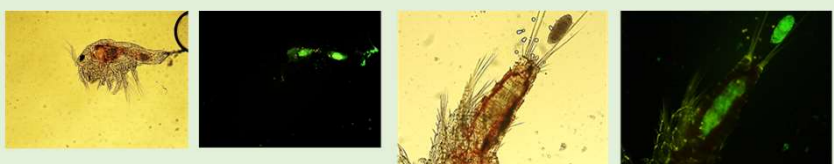


Fig. 5. *Tigriopus californicus* copepodite photographed without fluorescent light (left) and with fluorescent light (right). The copepodite has *Pichia pastoris* GFP yeast cells in its digestive tract.

Fig. 6. *Tigriopus californicus* copepod photographed without fluorescent light (left) and with fluorescent light (right). The copepod has *Pichia pastoris* GFP yeast cells in its digestive tract. It is clear that the yeast cells are a part of the intestinal contents as they are released as faeces, when they have gone through the digestive tract.

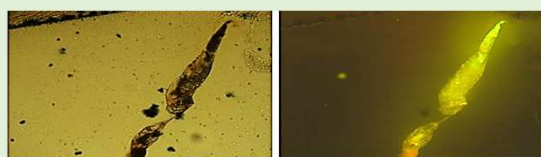


Fig. 8. The intestine of a whitefish larva photographed without fluorescent light (left) and with fluorescent light (right). The whitefish larva has *Pichia pastoris* GFP yeast cells in its digestive tract.

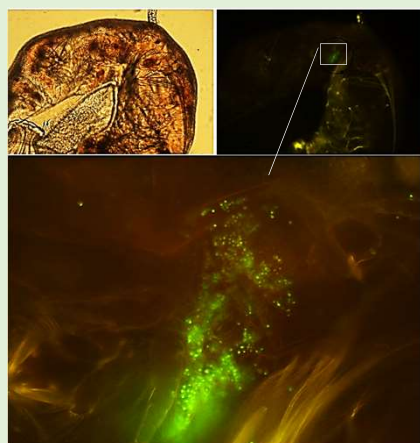


Fig. 9. The intestine of a flounder larva photographed without fluorescent light (left) and with fluorescent light (right). The flounder larva has *Pichia pastoris* GFP yeast cells in its digestive tract.

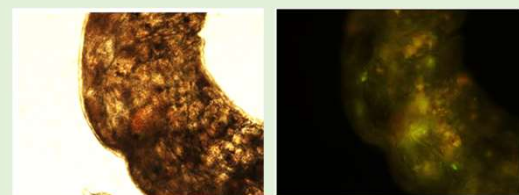


Fig. 10. The intestine of a flounder larva photographed without fluorescent light (left) and with fluorescent light (right). The flounder larva has *Yersinia ruckeri* GFP bacteria cells in its digestive tract.

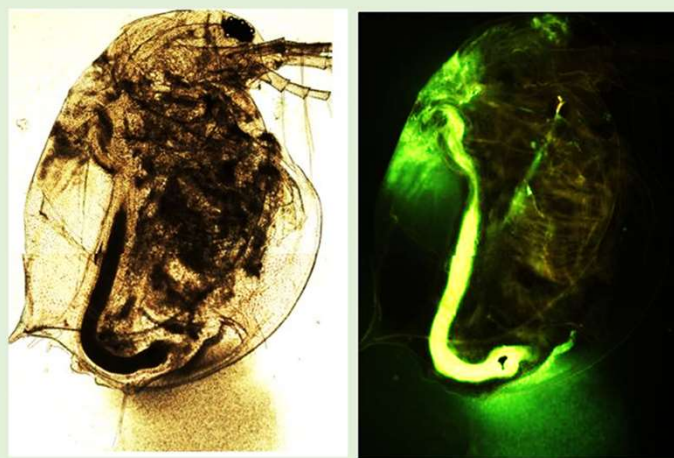


Fig. 7. *Daphnia magna* photographed without fluorescent light (left) and with fluorescent light (right). The daphnia has *Pichia pastoris* GFP yeast cells in its digestive tract.

Results

After feeding copepods, rotifers and *Daphnia* with *Pichia*-GFP, we could identify fluorescent yeast inside the organisms.

More importantly, the antigen was stable enough in the microorganism to be detected inside the fish larvae intestine fed with those GFP-microorganisms. Hence, we could express an antigen in yeast and transfer it into a prey organism.

After feeding rotifers with the bacterin *A. salmonicida*, unfortunately, the GFP signal in rotifers was absent except for one experiment. After feeding rotifers with the bacterin *Y. ruckeri* GFP, the GFP signal was present for all protocols tested (various temperature, light and feeding regimes).

Conclusions

The rotifers accumulated the fluorescent bacteria in their digestive tract, and could be used to deliver bacterin-based vaccines to fish larvae (fig. 1 to 3).

We found that all stages of *Daphnia* and copepods (from nauplii to adult) accumulated yeast in their digestive system. Yeast cells (*P. pastoris*) may be genetically modified to express various vaccine antigens derived from fish pathogens, hereby giving the approach a generic dimension (fig. 4 to 7).

Following feeding of the GFP-loaded crustaceans to fish larvae/fry, the fluorescence-loaded prey was found accumulated in the digestive tract of fish larvae (whitefish, flounder and rainbow trout), although the fluorescence signal was somewhat reduced. (fig. 8 to 10).