**Pichia pastoris yeast as a vehicle for oral vaccination of larval and adult teleosts**

Oral vaccination is of major interest because it can be used for mass vaccination of fish of various size and age. Given that their administration is relatively easy and stress-free, oral vaccines have both economic and animal welfare benefits. Yet, mostly due to their limited efficacy, only very few oral vaccines are available to aquaculture industry. Here we present a method for oral vaccine delivery based on the yeast Pichia pastoris. We could express a model antigen, green fluorescent protein (GFP), in this yeast and subsequently show delivery of the GFP protein to the intestine of juvenile flounder or adult carp and trout. We tested this approach in several commercially-relevant fish species, from juvenile to adult stage. To test the oral delivery of antigen to larval fish, the GFP-expressing Pichia pastoris was first fed to planktonic crustacean Daphnia or rotifers that served as 'bioencapsulation vehicles' and afterwards, fed to flounder larvae. Again, we could show delivery of intact GFP protein to the intestine. In rainbow trout, the orally-administered GFP-expressing yeast elicited a rapid local innate immune response in the intestine and a subsequent systemic response in the spleen. Our results show that Pichia pastoris is a good vehicle for oral antigen delivery and that it can be used in non-encapsulated form for older fish or in bioencapsulated form for larval fish. We discuss the immunomodulatory properties of the yeast itself, and its potential to enhance local immune responses and act as an adjuvant.

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