Abstract

Currently, one of the most significant risks to the fishing industry and salmonid fish farming is fish orthoreovirus (PRV) infection. In Europe, the most abundant subtype is currently PRV-3, which mainly attacks rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) and causes great economic and ecological issues. This virus causes a disease similar to HSMI (heart and skeletal muscle inflammation), which is triggered by its related subtype PRV-1. As the name suggests, the disease mainly affects the heart and skeletal muscles.

DNA vaccines are, thanks to their relatively undemanding and quick preparation, an increasingly popular variant of vaccination in the veterinary industry. Therefore, we used Gibson cloning to construct a total of 20 vaccine plasmids that contain genes for different viral proteins (σ1, σ3, μ1). At the same time, we combined these genes with different fusion partners and signal sequences to increase the effectiveness of the vaccine. This involved ensuring antigen secretion into the intercellular space or anchoring the antigen to the plasma membrane of the transfected cell.

Subsequently, we tested and confirmed the production of antigens (σ1, σ3) in the transfected HEK 293T cell line. We were also able to transfect the fish cell line RTGill-W1, in which no successful transfection has been reported so far. We were also able to identify the viral subtype in the infected blood sample we received from the affected area in Norway as PRV-1. We introduced an RT-qPCR method for its quantification as well.

This master's thesis was created in cooperation with the Czech-Danish company Dyntec spol. s.r.o. (Pražská 328, 411 55 Terezín), which focuses mainly on the development of veterinary vaccines and medicines.

Key words: PRV3, DNA vaccine, rainbow trout, Atlantic salmon, fish immunity