

The role of chitosan nanoparticles as a potential antibacterial agent and immune stimulant against fish pathogens

Chitosan [α (1 \rightarrow 4) 2-amino 2-deoxy β -D-glucan] is a naturally occurring biocompatible, biodegradable, non-toxic polymer. It is a partially de-acetylated polymer of chitin, which is a natural water insoluble linear polysaccharide polymer found abundantly in fungi and shells of crustaceans and insects. Chitosan is soluble in aqueous solutions (acidic media) so it is easily excreted from the kidney, therefore, chitosan and its derivatives are widely used in medical and pharmaceutical applications. The presence of primary amine groups in the repeated units of chitosan grants it several properties such as antibacterial, antifungal and antitumor activities. Nanoparticles are solid particles ranging from 1 to 1000 nm providing a bigger surface area for attachment of bioactive ingredients and a short diffusion path for substrates. Chitosan nanoparticles (CSNPs) were used as bioactive ingredient carriers, encapsulators or immobilizers in several drug and gene delivery systems. Recently, CSNPs exhibited antimicrobial activities against various pathogens *in vitro*, inhibiting the growth of several bacterial strains. More recently, CSNPs was used *in vivo* as immunomodulator to enhance growth and meat quality of tilapia and increase survival rates of crayfish challenged with white spot syndrome virus. Besides, CSNP induced several immune related genes in fish tissues during *Aeromonas hydrophila* infection. *Aeromonas spp.* are gram negative, straight rod pathogens that affect marine and freshwater fish species, including *Cyprinus carpio*. *Aeromonas salmonicida* subsp. *salmonicida* causes lethargy, lack of appetite, hyper-pigmentation and furuncles or ulcers on the skin, exophthalmia, septicemia, ascites, anaemia and haemorrhagic lesions in gills, fins, muscles, and internal organs. Recently, silver nanoparticles have shown promising results against *A. salmonicida* subsp. *salmonicida* *in vitro* and *in vivo*. Nevertheless, safety concerns have been raised over the usage of Ag-NPs because they may pose potential hazards for health and the environment. Therefore, a comparative *in vitro* study will be conducted to investigate the antimicrobial effects of chitosan and CSNPs against 5 selected bacterial pathogens. Depending on the results from the *in vitro* study, *in vivo* studies will be conducted to assess the prophylactic and therapeutic effects of dietary CSNPs by evaluating various fish immune parameters. In addition, proteomic approaches will be used to identify differentially expressed proteins in fish tissue. Biological functions and networks of the proteins will be investigated. Besides, mRNA expression levels of differentially expressed proteins will be measured by q-RT PCR at transcriptional levels. This study will give rise to the first proteomic profiles of *Cyprinus carpio* exposed to CSNPs.