

Pathogenicity of an Invasive Bacterium, *Aeromonas salmonicida* in Indian Major Carp, *Labeo rohita* – Evaluation of Immune Response Using Effective Molecular Markers

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Abstract

Effects of *Aeromonas salmonicida* on the non-specific immune response, oxidative stress and subsequent cell death in liver and spleen of *Labeo rohita* exposed to asymptomatic dose (2×10^7 CFU/mL) of pathogen were studied. Evaluation of in vivo non-specific immunity via Nitro blue tetrazolium (NBT) reduction assay, myeloperoxidase (MPO) assay and serum bactericidal activity assay exhibited significant alterations among infected fishes, indicating temporary surge of innate immune responses. Considerable cell death and damage was observed in the histopathological analysis of liver and spleen in the infected fishes. Flow cytometric analysis by FITC-Annexin V/PI conclusively indicated triggering of apoptotic cell death in both organs. Relative expression of pro-inflammatory cytokine (TNF α) gene, antimicrobial protein producing genes (Lysozyme C and Lysozyme G) and apoptosis effector molecule (Caspase 3) gene were increased significantly both in liver and spleen of *A. salmonicida* treated fishes. In conclusion, infection with *A. salmonicida* at asymptomatic level resulted in severe oxidative stress that, caused apoptotic cell death. Since facultative pathogen eradication is not possible in open water or flow-through aquaculture systems, this remains a crucial area of scientific research and the results obtained in the present study might provide better understanding of prophylactic, diagnostic and therapeutic measures during aquaculture.

Introduction

Aquaculture industries worldwide frequently suffer heavy financial losses that threaten their growth and sustainability, mainly due to uncontrolled microbial diseases resulting in mass mortalities (Almeida et al., 2009). The most significant factors out of all the microbial diseases are bacterial (Zorilla et al., 2003) and viral (Crane, & Hyatt, 2011). The study of fish diseases requires a wide knowledge, not only of the potential pathogens, but also of the environmental constraints and special adaptations, which govern the inflammatory and immune responses of fishes. Expedient recognition,

treatment and prevention of fish diseases are essential for the improvement of fish culture and subsequent increase in fish resources. The increased frequency of international transfer of exotic fish species in the last few decades have led to introduction of numerous novel and exotic pathogens in many countries (Arthur, 2005). In the Indian perspective, one such key invasive bacterial pathogen is *Aeromonas salmonicida*. According to Evenberg and Lugtenberg (1982) the virulence factor of *A. salmonicida* is a protein layer, composed of 50 kD protein (A-protein) known as A-layer. Although it was previously reported to cause disease exclusively in the salmonids, several cases of *A. salmonicida* infections