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ACCEPTED MANUSCRIPT

Cytotoxic Effects of *Aeromonas hydrophila* Culture Supernatant on Peripheral Blood Leukocytes of Nile Tilapia (*Oreochromis niloticus*): Possible Presence of a Secreted Cytotoxic Lectin

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Abstract

Number of exotoxins like haemolysin, leukocidin, aerolysin etc. were reported from *Aeromonas hydrophila*. In this study, we report the haemolytic and cytotoxic effect of *A. hydrophila* culture supernatant (CS) that is specifically inhibited by lactose and also by serum and mucus of Nile tilapia (*Oreochromis niloticus*). Hence, we assume the presence of a secreted lectin in the CS. CS is toxic to peripheral blood leukocytes (PBL) of *O. niloticus* as revealed by MTT assay and by flow cytometry. DNA laddering assay indicates that CS causes necrosis to PBL. As a result of necrosis, CS treated PBL showed increased production of reactive oxygen species as indicated by nitroblue tetrazolium and 2',7' –dichlorofluorescin diacetate assays. CS treated PBL showed reduced mRNA expression of TNF-α and IFN-γ genes. When CS was subjected to polyacrylamide gel electrophoresis, it showed a single band corresponding to the molecular weight of 45 kDa. However, upon concentrating the CS by ultrafiltration, many bands were visualized. Further studies at molecular level are required to unravel this macromolecular-leukocyte interaction which would ultimately benefit the aquaculture industry.

Keywords: Aquaculture; Exotoxins; Haemolysis; Lectin; Necrosis; ROS production

Introduction

Secreted exotoxins are one of the highly studied bacterial virulence factors (Alouf 2006). The virulence factors including exotoxins secreted by *Aeromonas hydrophila* were

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