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Cytotoxic effects of *Aeromonas hydrophila* culture supernatant on peripheral blood leukocytes of Nile tilapia (*Oreochromis niloticus*): Possible presence of a secreted cytotoxic lectin

Parasuraman Aiya Subramani [a], R. Vydyanath Narasimha [b], Ramalakshmi Balasubramanian [a], Venkata Ramireddy Narala [b], M.R. Ganesh [c], R. Dinakaran Michael [a]

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Highlights

- Cytotoxic activity of Aeromonas hydrophila culture supernatant (CS) was tested.
- CS lysed RBC and peripheral blood leukocytes (PBL) of *Oreochromis niloticus.*
- CS caused necrosis to *O.niloticus* PBL.

- Low concentration of CS significantly increased ROS production by PBL.
- CS downregulated expression of TNF-α and IFN-γ genes.

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.

Introduction

Secreted exotoxins are one of the highly studied bacterial virulence factors [1]. The virulence factors including exotoxins secreted by *Aeromonas hydrophila* were already reviewed by Ref.[56]. Most of these toxic virulence factors have implications in aquaculture [7], and human and animal health [28]. Most of these toxins were secreted by the type III secretion system which can impair NF-κB pathway [47]. Haemolytic lectins are proteins that lyse red blood cells by specifically binding to sugar moieties present in the cells' surface [31].

Lectins in general, act as a biological recognition molecule that plays critical role in host-pathogen interactions [45]. Haemolytic lectins have been isolated from different organisms that range from plant *Sterculia foetida* [8], Echinoderms, *Holothuria grisea* [34] and *Cucumaria echinata* [67] to snake *Daboia russelii* [35].

Lectins were also reported from different bacterial pathogens and shown to have important role in pathogenesis. For instance, *Pseudomonas aeruginosa* lectins, LecA and LecB play a vital role in biofilm formation [55] and adhesion to host cells [55], [21]. Cholesterol-dependent cytolysins, pneumolysin and streptolysin O were recently found to be lectins [46]. However, lectins present in *A.hydrophila* were not well studied. Lectins of many fish pathogens were found to facilitate adhesion to host cells. For instance, lectins of *Edwardsiella ictaluri* helps in adhering to the olfactory mucus of the host, *Ictalurus punctatus* [65].

Programmed cell death is thought to be an important counter-attack by host against certain pathogens [70]. Necrosis is often associated with the release of intracellular immunostimulatory compounds [62]. Some toxic lectins were shown to induce necrosis to various cell lines *invitro* [3]. Necrotic death is routinely studied by the characteristic smear of genomic DNA, when DNA isolated from necrotic cells is subjected to electrophoresis [14], [42]. *A.hydrophila* has been reported to cause necrosis of soft tissues [60].

Despite disease-outbreaks and economic losses [66], tilapia (*Oreochromis* sp.) is widely cultured world-wide [54]. Fishes possess a primitive immune system in which the skin mucus is one important physical barrier against infection and immune related compounds present in serum (humoral) are comparable to those of higher vertebrates [30]. Molecules present in biological fluids like serum that recognize and neutralize secreted toxins were considered to be an important therapeutic agent in humans [68].

Teleost fishes possess a well-developed cytokine signalling system comparable to that of mammalian counterparts [64]. Type II interferons are well characterized in several fishes [71]. Lectins modulate the immune-reactive cells by regulating the expression of cytokines [59].

In the present study, we suppose the possible presence of a lactose specific lectin among the exotoxins secreted by *A.hydrophila* in the culture supernatant (CS). Interestingly, in addition to lactose, haemolytic activity of CS was inhibited by serum and skin mucus of Nile tilapia, *Oreochromis niloticus*. This indicates that lectin present in CS could possibly interacted with these body fluids of *O.niloticus.* Further, CS causes necrosis of peripheral blood leukocytes (PBL) and downregulates the expression of genes encoding tumour necrosis factor alpha (TNF-α) and interferon gamma (IFN-γ). This interaction may have implication in pathogenesis of *A.hydrophila* in this fish. Further studies should be done to purify the causative toxic molecule and further find out the molecules that neutralize the toxin in order to find a good therapeutic agent for tilapia.

Section snippets

Bacterial strains and culture conditions

A virulent strain of *A. hydrophila* was collected by peritoneal lavage from a diseased *Oreochromis niloticus* in this laboratory during the period of April-May, 2014. The diseased fish showed symptoms like severe haemorrhage all over the body, distended abdomen etc. Upon autopsy, the visceral organs were found to be disintegrated. The Bacterium was cultured in trypticase-soy (TS) broth at 28 °C with 200 rpm in an orbital shaking incubator (Labnet, Chennai). Only second passage of bacteria was

Bacterial strains

Isolated bacteria gave positive results to the biochemical tests for *A. hydrophila* (Supplementary Table 1). The bacterium was able to grow in *A. hydrophila* selective Rimmler-Shotts medium (Supplementary Fig. 1). Phylogenetic analysis of the 16S rDNA sequence shows high similarity to available sequences of *A. hydrophila* (Supplementary Fig. 2).

Haemolysis and haemolysis inhibition assays

We observed a time dependent lysis of cells. At first half an hour of incubation, literally there was no cell lysis followed by gradual lysis of RBC

Discussion

Protein haemolysin is an important exotoxin and virulence factor for many pathogens [9]. Some of the haemolytic [16] and cytotoxic proteins [18], [10] were already isolated from *A. hydrophila.* In this study, we report the haemolytic properties of CS. When culture supernatant was heated at 60 °C for 30 min, haemolysis was inhibited (data not shown). This

indicates the heat labile protein nature of the haemolytic lectin present in the culture supernatant.

Lactose, a disaccharide inhibited the

Conclusion

In this study we report the haemolytic and cytotoxic properties of CS. CS is toxic to immune cells and recognized by body fluids including serum and skin mucus. CS also causes necrosis of cells. These properties of CS may be attributed to the possible presence of a lactose specific haemolytic lectin present in it. Hence, this study could be used as a lead to further dissect the pathogenesis mechanisms by *A.hydrophila* at a molecular level using advanced proteomic and genomic tools.

Acknowledgements

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> …ROS and RNIs have also microbursts that inflict oxidative destruction of the pathogen leading to the incitation of indispensable enzymes [74]. In Oreochromis niloticus the heat-killed or supernatant of A. hydrophila improved the synthesis of ROS by PBL [52,75]. In the present study, the ROS production is significantly high in the healthy group when fed with 10 mg kg−1 CM diet; both groups fed with 15 mg kg−1 CM diet had enhanced ROS on 2nd and 4th weeks.…

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Silver nanoparticles mitigate Aeromonas hydrophila-induced immune suppression, oxidative stress, and apoptotic and genotoxic effects in Oreochromis niloticus

2021, Aquaculture

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> …It produces virulence factors, such as hemolysins, aerolysins, adhesins, enterotoxins, phospholipases, and lipases (Stratev and Odeyemi, 2016) that cause macroscopic and microscopic degenerative changes (El Deen et al., 2014Yardimci and Aydin, 2011). Moreover, the increased production of reactive oxygen (Subramani et al., 2016) in response to reactive oxygen species (ROS) accumulation activates the NF-kB pathway (Banerjee et al., 2014). Silver nanoparticles (at a concentration of up to 5 μg/mL) have been reported to significantly reduce the growth of all studied isolates of Streptococcus iniae, Lactococcus garvieae, A. hydrophila, as well as Yersinia ruckeri, after 30 to 90 min of exposure or at least minimize the disease outbreaks (Soltani et al., 2009).…

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> …The intensity of bands was analysed using ImageJ software [41]. Data were then presented as ratio of expression (relative expression) of gene of interest to that of the expression of β-actin [20]. Data were presented as mean±SEM.…

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Expression, purification and biological characterisation of recombinant human irisin (12.5 kDa)

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> …One hundred micro litres of purified rh-irisin (0.5 mg/ml) was loaded on analytical HPLC (LC-2010C HT® system, Shimadzu Corporation, Kyoto, Japan) connected to reversed phase C4 analytical column (150 × 4.6, 5 µ particle size, 30 nm pore size; Grace Vydac) using solvent A (0.1% TFA) and solvent B (90% acetonitrile with 0.1% trichloroacetic acid) with linear gradient of B composition (0 to 30 min: 36–55% of B, 30 to 35 min: 56–100% of B; 35 to 45 min: 100% of B and 45 to 50 min: 100–36%, 50 to 60 min 36%) with flow rate of 1.2 ml/min, samples were detected at 214 nm. The effect of E. coli derived rh-irisin on 3T3-L1 cell proliferation was determined by measuring the activity of mitochondrial dehydrogenase-enzyme of living cells, where the enzyme converts the tetrazolium bromide (MTT) into a purple formazan product and the intensity of the colored product was measured by using spectrophotometry as described previously [9]. Briefly, 3T3-L1 cells (3 × 105 cells/ml) were cultured in DMEM medium containing 10% FBS, 100 U penicillin/ml, 100 µg/ml streptomycin (Gibco) and incubated at 37 °C for 24 h with 5% CO2 in 96 well culture plate.…

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