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Changes in serum electrophoretic profile of *Lutjanus analis* (Cuvier, 1828) in response to acute infection by *Listonella* (syn. *Vibrio*) *anguillarum*

Cambios en el perfil electroforético sérico de *Lutjanus analis* (Cuvier, 1828) en respuesta a la infección aguda por *Listonella* (*sin. Vibrio*) *anguillarum*

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ABSTRACT | The results of electrophoresis of the blood serum of *Lutjanus analis* in agarose gel from two groups of fishes: (a) fish exposed to live *Listonella anguillarum* diluted in 0.9% NaCl solution (saline) by intramuscular injection and (b) control specimens injected with the saline solution are provided. Differences in the serum protein profiles of the two groups of specimens were observed. The method here employed, seems to be useful in detecting potential markers for monitoring infection or inflammatory reactions in *L. analis* after acute infection by *L. anguillarum* and has the advantage to allow easy analysis of several samples in a short time.

Palabras clave
Proteinograma
Pargo
Vibriosis
Diagnóstico de enfermedades

RESUMEN | Se proveen los resultados de la electroforesis del suero sanguíneo de *Lutjanus analis* en gel de agarosa de dos grupos de peces: (a) peces expuestos a *Listonella anguillarum* vivo diluido en solución de NaCl al 0,9% (solución salina) por inyección intramuscular y (b) peces del grupo control inyectados con solución salina. Se observaron diferencias en los perfiles de proteínas séricas de los dos grupos de muestras. El método aquí empleado parece ser útil para detectar marcadores potenciales para monitorear la infección o reacciones inflamatorias en *L. analis* después de una infección aguda por *L. anguillarum* y tiene la ventaja de permitir analizar fácilmente varias muestras en poco tiempo.

INTRODUCCIÓN

The most recent statistics from FAO indicate that world aquaculture production reached a historical record of 114.5 million tonnes live weight in 2018 with a total sales value of 263.6 billion USD, dominated by finfish from continental marine and coastal aquaculture (Zhou, 2020). Furthemore, data from the Global Aquaculture Alliance survey for key fish species presented at the GOAL 2019 annual conference showed that farmed fish production increased 73% in the last decade (Tveteras *et al.*, 2020). Although the strategy to increase the productivity of aquaculture is based on the development and maintenance of intensive farming systems, this can have adverse consequences, mainly because it requires maintaining high population densities in confinement, which promotes the appearance of disease outbreaks that constitute a real problem for the growth of aquaculture production (Adams *et al.*, 2008).

Farmed fish are exposed to changes in environmental variables, management, aspects of nutrition and food, and stress that lead to the generation of diseases (Bondad-Reantaso *et al.*, 2005, Bowater *et al.*, 2012, Albert and Ransangan 2013). Among fish bacterial diseases, vibriosis is an opportunistic pathology transmitted mainly by *Listonella anguillarum* (formerly *Vibrio anguillarum*), a gram-negative halophilic bacterium whose effects include deadly hemorrhagic septicemia. Vibrosis affects more than 50 fresh and salt-water fish species including various species of economic importance in the aquaculture industry (Frans *et al.*, 2011, Ina-Salwany *et al.*, 2019). Due to its high morbidity and mortality rates, this disease is responsible for serious economic losses worldwide, both in aquaculture and in larviculture. Therefore, fast,

accurate, and reliable detection of vibriosis is essential to take adequate control measures that effectively reduce the economic losses caused by this pathology (Frans *et al.*, 2013).

The mutton snapper, *Lutjanus analis* (Cuvier, 1828) is an economically important finfish belonging to the family Lutjanidae with the potential for commercial aquaculture (Watanabe *et al.*, 1998, Watanabe 2001). This species is distributed along the Western Atlantic as far north as Massachusetts, USA, Bermuda, and southward to southeastern Brazil, including the Caribbean Sea and the Gulf of Mexico (Froese and Pauly, 2020). Experimental studies on the cultivation of this species showed that it is a good candidate for farming due to its adaptability to captivity and rapid growth on diets containing high levels of high-quality plant protein sources such as soy protein concentrate (Botero and Ospina, 2002; Freitas *et al.*, 2011; Alvarez-Lajonchère and Ibarra-Castro, 2013) thus producing high-quality meat. Thus, efforts contributing to the knowledge of its biology are important for the use of this fish as a crop species.

In this framework, we investigated the response to the acute infection caused by *Listonella anguillarum* in *L. analis*, aimed to identify a rapid marker for the screening of fishes that allows the identification of infected fishes in farming conditions. The serum electrophoretic profile of individuals exposed by injection with a suspension of live bacteria was compared with those of non-exposed individuals.

The fish studied here were caught near the inlet channel of Laguna La Restinga (10°58'23.5"N 64°10'21.6"W), Isla de Margarita, Venezuela, using a 50 m long seine net and confined in a circular concrete pond with constant aeration and continuous flow of seawater, where the fish were kept for 15 d. During this time, the fish were fed with live pupfish (*Cyprinodon dearborni*) and sardines (Engraulidae). The experiment followed the ethical/anesthesia conducts and was approved by the Ethics Committee on Animal Experimentation of the Universidad Técnica de Machala (Protocol number UTMACH-CEEA-006-2018-AC).

Cells of *L. anguillarum* were grown in tryptic soy broth (TSB) containing 1.5% NaCl and washed three times with saline solution (0.9% NaCl) before being inoculated. A group of fish (n=10; with average weight 70.8 ± 12.6 g), was injected intramuscularly (IM) with 0.2 mL of a suspension of live bacteria (10^9 ce1/ml) diluted in saline solution and bled when signs of the disease were evident, which occurred at 72 h after inoculation; the control group (n=10; 72.0 ± 12.7 g), was injected with saline solution. Blood samples were collected from each specimen under anesthesia with 100 mg/ MS-222, by venipuncture with sterile hypodermic 21 G needle in, the midline just posterior to the anal fin by inserting the needle at a 45˚ angle into the musculature, perpendicular to the ventral surface of the fish until reaching the spine or blood entered the syringe. Fish from the control group were allowed to recover by placing them in fresh, aerated seawater and released the next day after checking their health. Fish inoculated with *L. anguillarum* were euthanized with an anesthetic overdose and incinerated.

After collection, the whole blood was kept at room temperature and after 30 minutes, the clot was removed by centrifuging at 4000 rpm for 5 min. The serum samples were withdrawn with Pasteur pipet, placed in Eppendorf tubes, and frozen (-20° C) until use. One µL of each sample was applied to 1.5% agarose gel in 0.05 M barbital buffer, pH 8.6 and electrophoresed at 90 volts (30 mA) for 35 min; the gels were subsequently fixed in a 5% acetic acid solution for 5 min and stained with a solution of 0.5% Ponceau Red in fixative for 5 min. Gels were washed with fixative for to destain and then dried at 65°C for 30 min. The protein profiles were obtained in a fluorometer/densitometer (Corning-710) at 520 nm. A sample of normal human serum was used for comparisons.

Electrophoresis of human serum proteins showed the typical six well-defined bands (Fig. 1a); those of the control fish group consistently revealed six main fractions that were labeled in order of decreasing electrophoretic mobility (I to VI) (Fig. 1b) that corresponded to the mobility and spatial relationship of albumin, α1, α2, β1, β2 and γ globulins from normal human serum.

Fish injected with the live bacteria, showed significant changes in the electrophoretic proteinogram compared to those of the control group. Indeed, besides the six main fractions two additional ones (VII and

VIII) were identified, much more cathodic than the human γ globulins (Fig. 1c). The appearance of these two new fractions coincides with the behavior of acute phase serum proteins (APP).

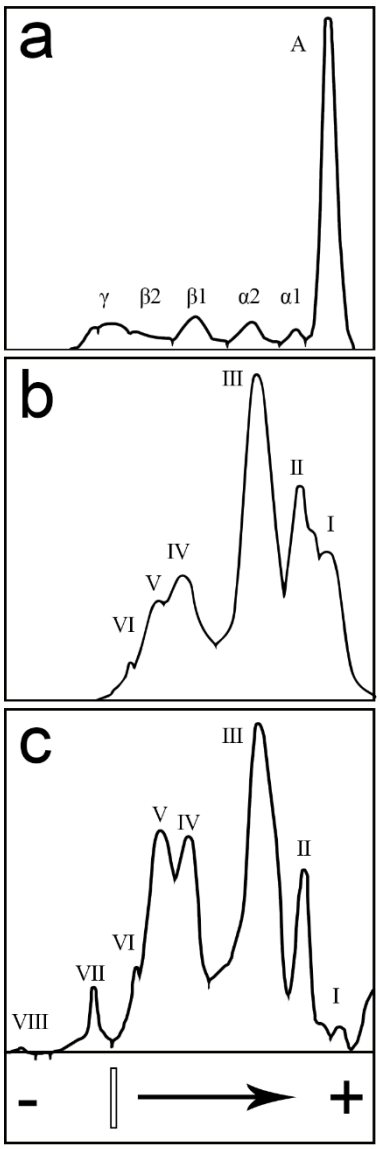

Figure 1. Electrophoretic profile of human serum (a), control (b) and infected (c) snapper serum in agarose gel. In (a) Albumin (A) and α1, α2, β1, β2, and γ globulins in order of anodic mobility. In (b-c) Roman numerals indicate the protein fraction of *L. analis*. The arrow indicates the direction of migration.

APP are glycoproteins that can have various functions, such as opsonization, activation of the complement system, and modulation of the host immune response (Gruys *et al.*, 2005); they are released into the circulation in response to external stimuli or tissue injury, and their concentration changes after infection, inflammation, trauma, or stress (Murata *et al.*, 2004). The APPs characterized in mammals have been indicated to be homologous to proteins identified in fish species and are believed to perform similar functions, although the mechanisms of induction of the synthesis of these proteins in fish are not clearly understood (Christiansen *et al.*, 2015).

Significant changes in APP have also been observed in *Oncorhynchus mykiss* after the injection of a *Vibrio anguillarum* bacterin emulsified in Freund's incomplete adjuvant (Gerwick *et al.*, 2002), although these results were obtained by polyacrylamide gel electrophoresis which is more laborious and time-consuming than that performed on agarose gels. Thus, detecting changes in the protein fraction of the electrophoretic profile on agarose gels seems to be useful as a potential method of monitoring the induced changes during infection or inflammatory reactions in *L. analis* after acute infection by *L. anguillarum.*

Furthermore, this method for serum analysis is relatively simple and cheap and allows to easily analyze many samples in a short time.

Conflict of interest

Authors declare no conflict of interest

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