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FISH PASTEURELLOSIS: SENSITIVITY OF WESTERN BLOTTING ANALYSIS ON THE INTERNAL ORGANS OF EXPERIMENTALLY INFECTED SEA BASS (*DICENTRARCHUS LABRAX*)

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Abstract

In this study we evaluated the sensitivity of western-blotting in detecting system bacterial infections caused by *Photobacterium damsela* subsp. *piscicida* in internal organs of experimentally infected sea bass. Western blotting revealed cross-reactivity in liver homogenate only, but not in spleen and serum.

Introduction

Fish pasteurellosis caused by *Pasteurella piscicida*, now *Photobacterium damsela* subsp. *piscicida* (Gauthier *et al.*, 1995) is one of the main bacterial diseases causing severe losses in marine and euryhaline fish farms.

There are several studies on the immunological aspects of fish pasteurellosis: the aim of this work is to evaluate the sensitivity of western-blotting in detecting bacteriosis caused by *Photobacterium damsela* subsp. *piscicida* in internal organs of experimentally infected sea bass (*Dicentrarchus labrax*).

Materials and Methods

For the experimental infection of 70g sea bass, a virulent strain of *Photobacterium damsela* subsp. *piscicida* (*Pasteurella piscicida* 945/93/UD, Istituto Zooprofilattico Sperimentale delle Venezie, Dipartimento di Ittiopatologia, Basaldella di Campoformido, UD, Italy) was used.

Fish stocks were determined to be free of this pathogen by common microbiological analysis of internal organs.

Fish were infected by immersion (20 fish) or by intraperitoneal injection (i.p.) (20 fish).

Immersion infection was performed in 10 litre aquaria with a bacterial concentration of approximately 10^8 cfu/ml (colony forming unit/ml); fish were maintained 2 h in this bath and then kept into 500 litres aerated recirculating water at 26‰ salinity and 26°C. Control fish were maintained in an identical bath without bacterial suspension.

The i.p. infection was performed by injecting 0.2 ml of 10^6 cfu/ml of *Ph. damsela* subsp. *piscicida* in sterile PBS; control fish were injected with sterile PBS only.

Spleen and liver of dead fish were removed immediately, homogenised in cold 1:5 PBS (w/v) with an Elvejem potter and then frozen in cryotubes at -80°C. In moribund fish, where possible, serum protein content was determined according to Lowry *et al.* (1951) using serum albumin as a standard reference.

SDS-gel electrophoresis was performed using the discontinuous system of Laemmli (1970) with 3% and 12% acrylamide in the stacking and separation gel, respectively. For Western-blotting proteins were transferred from the slab gel to nitrocellulose filters following the method of Towbin *et al.* (1979).

Immunodetections were performed using polyclonal anti- *Ph. damsela* subsp. *piscicida* serum (1:200 dilution) as primary antibody and IgG anti-rabbit peroxidase conjugate as secondary antibody, the development was performed with 4-chloro-naphthol. As positive standard a frozen 10^9 cfu/ml *Ph. damsela* subsp. *piscicida* PBS suspension was used.

The polyclonal antibodies (Poly Ab) were obtained by injecting intramuscularly and subcutaneously rabbits with saline-washed suspension (10^9 cfu/ml) of formalin killed

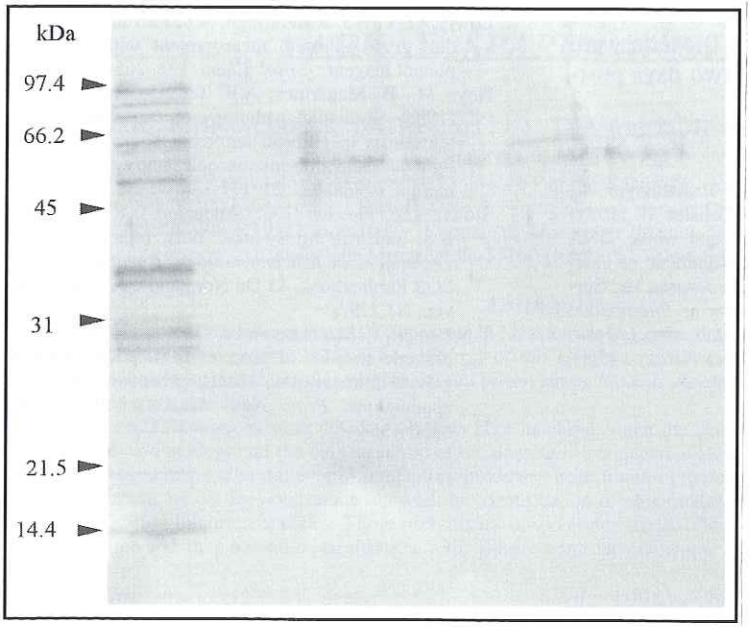

Figure 1 Western blot of liver homogenate from control and immersion infected fish 2 days post challenge. Homogenates (50mg of protein per lane) and *Ph. damsela* subsp. *piscicida* suspension (20mg protein) followed by immunoblot using polyclonal anti- *Ph. damsela* subsp. *piscicida*. Lane 1 contained the *Ph. damsela* subsp. *piscicida* suspension, lane 2 empty, lane 3 liver homogentate of control fish, lanes 4, 5 and 6 liver homogenates from infected fish.

bacteria (whole cells) according to Stolen *et al.* (1990).

Results

All fish infected (by immersion and i.p.) died after within two days.

In some fish (10 and 6 in i.p. and immersion trials respectively) was possible recovering pure culture from internal organs.

The frozen *Pasteurella piscicida* suspension shows cross-reactivity bands at various molecular weight: 97.4, 93.7, 90, 62, 57.7, 36.7, 34.3, 29.7 and 27.1 kDa.

The electrophoresis analysis of liver and spleen homogenate in control and infected sea bass (either by immersion or by i.p.) gave similar electrophoretic patterns with differences in the intensity of the bands (data not shown).

In the liver homogenate from i.p. infected sea bass bands were evident between 97.4 and 45 kDa and also at lower molecular weight (M.W.), ranging 21.5 to 31 kDa, whereas in liver obtained from immersion infected sea bass, the electrophoretic pattern showed bands from 97.4 to 45 kDa and around 31 kDa.

Western blotting analysis of liver from sea bass infected by immersion with *Photobacterium damsela* subsp. *piscicida* is shown in Figure 1.

Poly Ab showed a cross-reaction at high M.W. from 64.8 to 41.4 kDa, and the reaction resulted similar in all infected sea bass.

Control and infected sea bass showed quantitative differences in bands: in fact, Poly Ab reaction recognised one band at 56.3 kDa, moreover in infected fish were possible recognise other three bands of about 41.4, 59.1 and 64.8 kDa.

The same pattern was found in i.p. infected sea bass.

We performed western blotting also on spleen homogenates and sera of control and infected (i.p. and immersion) fish but were no possible recognise cross-reactivity signals with Poly Ab.

Discussion

The present study showed that western blotting revealed cross-reactivity on liver homogenate only, but not in spleen and serum: probably, after two days post infection, only in the hepatic tissue high level of bacterial proteins were reached and so signals can be revealed by western blotting.

On the contrary, Noya *et al.* (1995) after 6 h post-i.p. infection in gilthead seabream (*Sparus aurata*) observed (by microscopy) bacteria in kidney and spleen. Bacteria were also observed in liver at 1 or two days post-infection.

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