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Response and function of cutaneous mucosal and serum antibodies in barramundi (*Lates calcarifer*) acclimated in seawater and freshwater

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Abstract

Mucosal and serum antibody responses were studied in sibling barramundi (*Lates calcarifer*) acclimated in either seawater or freshwater following vaccination by intraperitoneal injection or direct immersion in an inactivated *Streptococcus iniae* vaccine. As expected, route of vaccination had a marked effect on immune response, with direct immersion resulting in low serum antibody levels against *S. iniae* by ELISA detected 21 days post vaccination at 26 °C, whilst a significant response was detected in mucus. A strong specific antibody response was detected in both mucus and serum 21 days following intraperitoneal injection. Fish acclimated in seawater prior to vaccination showed a markedly higher specific mucosal antibody response than sibling fish acclimated in freshwater, regardless of the route of vaccination, whilst the serum antibody response was not affected by salinity. Both mucosal and serum antibodies from fish in seawater and freshwater were capable of binding antigen at salinities similar to full strength seawater in a modified ELISA assay. These results indicate that this euryhaline fish species is not only able to mount significant specific antibody response in cutaneous mucus, but that these antibodies will function in the marine environment.

Keywords: Vaccine; Mucosal antibody; Binding affinity; Salinity; Asian sea bass; Barramundi

1. Introduction

Asian sea bass or barramundi (*Lates calcarifer*) are a euryhaline fish cultured in tropical and subtropical environments throughout the Asia-Pacific region. Teleost fish are in continuous contact with their environment via their mucosal epithelium and this raises some interesting questions with respect to mucosal immunity, particularly in the case of euryhaline species in which the salinity of their environment can vary dramatically.

Teleost fish have been shown to have an effective mucosal immune system [1–3]. Mucosal immunity in teleost fish has received some attention in terms of kinetics and effects of routes of immunisation [1–5]. It has also been suggested that, in common with mammalian systems, different immunoglobulins may be associated with the mucosal

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response in carp, *Cyprinus carpio* [4] whilst a single isotype may be associated with mucosal immunity in pufferfish, *Takifugu rubripes* [5].

The effect of salinity on immune parameters has been less widely studied. In gilthead seabream, *Sparus aurata*, total plasma immunoglobulin levels increased in hypersaline water compared to seawater or low salinity water [6]. Other studies have focused on the effect of transfer of salmonid species from freshwater to seawater, generally indicating decrease in non-specific immune parameters, and attributing this to stress associated with shock [7].

Bricknell et al. [8] reported that Atlantic salmon, *Salmo salar*, antibodies did not function at extremes of pH and salinity. Indeed in their study, they demonstrated that specific antibodies against a model antigen, mannose-binding protein, were unable to bind in an ELISA at salinities equivalent to full strength seawater (1000 mOsm) [8]. The authors claimed that this study casts doubt on the ability of salmon antibodies to function in the gut or surface mucosae when in seawater and questioned the relevance of studies into vaccines against ectoparasites such as the sea louse [8]. This proposition would seem contradictory to evolutionary efficiency, as high levels of antibody are secreted into the mucosae in marine and euryhaline species [2]. Why would evolution select for this trait, requiring a high metabolic energy input into antibody secretion, if there was no beneficial advantage to be gained through inability to bind potential external pathogens?

In the present study, we set out to investigate the effects of salinity on the serum and mucosal antibody response and function in a euryhaline species, *Lates calcarifer*, acclimated in both fresh and seawater, in order to clarify the relevance of secreted antibody to this species in the marine and freshwater environment.

2. Materials and methods

2.1. Experimental animals

Sibling Asian sea bass (Barramundi) (*Lates calcarifer*), approximate weight 30 g were obtained from a commercial producer (Barramundi Australia, Stapylton, Qld, Australia) and held in brackish water at 10 ppt salinity in 60 L glass aquaria. Aquaria were organised into two banks of four tanks, each bank supplied by a separate recirculation system comprising a 300 L sump and 100 L biofilter. Fish were distributed such that there were eight individuals per tank and fish were initially maintained at 10 ppt salinity, 26 °C with recirculation adjusted such that each tank received approximately 120 L/h. Fish were fed a commercial 4 mm floating diet (Ridley Aqua Feed, Narangba, Qld) to satiation twice daily.

2.2. Preparation of vaccine

Streptococcus iniae strain QMA0087, isolated from diseased barramundi in Lake Argyle, Western Australia, and a kind gift of Dr Nicky Buller, was stored in the CMS strain collection in vegetable peptone broth + 20% glycerol at −80 °C. The strain was recovered from stock on Columbia agar base containing 5% defibrinated sheep blood. Identity was confirmed by API 20 Strep (BioMérieux, Marcy L'Étoile, France) and by PCR based on the lactate oxidase gene as described previously [9]. A single colony picked from an overnight agar plate culture was used to inoculate 10 ml vegetable peptone broth and incubated at 37 °C with shaking at 200 rpm for 24 h. An aliquot (0.5 ml) of this culture was then used to inoculate 2 × 2 L Erlenmeyer flasks, containing 500 ml vegetable peptone each and incubated with shaking (200 rpm) for 24 h. Final optical density at 600 nm was approx 1.60. The cultures were rapidly chilled on ice and formalin was added to a final concentration of 0.2% from a 40% stock. The formalinised bacterins were incubated at 4 °C with gentle agitation and an aliquot (200 μL) was taken at 24 h and 48 h onto blood agar to confirm inactivation of the culture. Bacterins were stored in sterile bottles until required.

2.3. Experimental design

The experiment was designed to determine if there is any difference in antibody binding affinities for mucosal and serum antibodies from euryhaline fish acclimated in fresh or salt water systems. Thus, after acclimation into the aquarium systems in brackish water, salinity in one system was progressively increased through daily 10% (v/v) water changes with full-strength (35 ppt) seawater. In the second system, salinity was progressively reduced by daily 10% (v/v) changes with fresh water. Specific gravity in the systems was checked daily with a hydrometer. After