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Ontogeny and modulation after PAMPs stimulation of β-defensin, hepcidin, and piscidin antimicrobial peptides in meagre (*Argyrosomus regius*)

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ABSTRACT

Antimicrobial peptides (AMPs), components of innate immunity, play an important role in protecting fish. In this study we report the molecular cloning of full open reading frames and characterization of expression of three AMP genes (*β-defensin* (*defb*), *hepcidin* (*hep2*), *piscidin* (*pisc*) in meagre (*Argyrosomus regius*). A phylogenetic analysis of the expressed sequences obtained shows the *defensin* isoform forms a clade with the other members of the beta class of this family, *hepcidin* corresponds to *hepcidin 2*, and *piscidin* corresponds to class I of its respective family. Gene expression profiles of AMPs was investigated, by means of quantification of mRNA in nine development stages, from 8 days post-hatching (dph) to accomplishment of juvenile form (120 dph). During development it was demonstrated *defb, hep2, pisc* were expressed in all stages of larval development and in juvenile tissues (kidney, spleen gut and gill). Moreover, expression patterns suggest the expression levels of theses AMPs are influenced by live prey (rotifer, *Artemia*) and first intake of commercial diet. Induction experiments *in vivo* (24 h) and *in vitro* (4, 12, 24 h) with PAMPs (LPS, poly (I:C), β-glucan) revealed significant changes in gene expression of the three AMP genes, in kidney, spleen, gut and gill. However, expression profiles differed in magnitude and time course response. *defb* expression shows a similar trend *in vivo* and *in vitro* in kidney at 24 h after LPS and β-glucan stimulation. The *hep2* expression levels were up-regulated upon β-glucan challenge *in vivo*, more in gut and gills than kidney, while *in vitro hep2* expression was up-regulated in kidney cells by LPS, poly (I:C), β-glucan (4 h). *pisc* expression was up-regulated in kidney cells, splenocytes by β-glucan, but in gill cells by poly (I:C) and β-glucan *in vivo*. However, *pisc* expression was upregulated in kidney cells by β-glucan and gill cells by LPS at 4 post-stimulation *in vitro*. These data suggest that AMPs play an important role in defense against pathogens, with each AMP having differing efficacies against specific types of microorganisms, although follow-up studies focusing on the biological activities in fish are needed.

1. Introduction

Antimicrobial peptides (AMPs) are an evolutionarily conserved component of the innate immune response and recognized as a critical first-line defense against many pathogens. These innate effector molecules are present in both vertebrate and invertebrate life forms and play an important role in protection against a broad spectrum of pathogens including those of parasite, bacterial, fungal and viral aetiology [1] by molecular mechanisms of cellular disruption [2]. Fish is continuously fights against pathogens by secreting a wide range of AMPs [3] They are typically present in leukocytes (mast cells, neutrophils), mucus cells (goblet and rodlet cells), cells lining epithelial surfaces and in gill, skin intestine and other tissues [4–7].

As a broad category of innate immune effector molecules, AMPs are divided into different families. Several studies have found peptides in a wide variety of species belonging to the defensin, parasin, cathelicidin and hepcidin families, as well as piscidin, a family unique to teleost fish [8]. Many of the peptides identified have antibiotic activity as well as immunomodulatory functions.

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