RECOMBINANT EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF A NOVEL ISOFORM OF HEPCIDIN FROM THE PONY FISH, *LEIOGNATHUS EQUULUS*

Aishwarya Nair[1*], Sruthy K. S[1]., Jayesh P.[1], I. S. Bright Singh[2], Rosamma Philip[1]

[1] *Department of Marine Biology, Microbiology & Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Fine Arts Avenue, Kochi 16, Kerala, India*

[2]*National Centre for Aquatic Animal Health, Cochin University of Science and Technology, Kochi-16, Kerala, India*

ABSTRACT

Hepcidins, are small cationic cysteine-rich amphipathic peptides with antimicrobial activity expressed mainly in the liver of living organisms and they play important roles in the host's immune response against microbial invasion and regulation of iron metabolism. Here, we report the recombinant production and functional characterization a novel HAMP-2 hepcidin isoform, *Le*-Hepc2 from the common pony fish, *Leiognathus equulus*. A 261 bp fragment cDNA with an ORF for 86 amino acids was obtained .The coding sequence encodes for 24 aa signal peptide coupled to a 36 aa prodomain and a 26 aa mature peptide. The mature peptide of *Le*-Hepc2 has a molecular weight of 2.73 kDa with a net charge of +2. Three dimensional structure of Le-Hepc2 mainly consists of two antiparallel β sheets stabilised by four di-sulphide bonds. The mature hepcidin was expressed as a 22 kDa fusion protein in *Escherichia coli*. Soluble recombinant peptide (r*Le*-Hepc2) containing a N-terminal hexahistidine tag was obtained from expression plasmid pET-32a+/*Le*-Hepc2 in Rosettagami B pLys S cells. It was purified by immobilized metal affinity chromatography (IMAC).The active purified recombinant hepcidin showed antimicrobial activity even at 5 μM against Gram-negative bacteria *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *Vibrio vulnificus* and *Edwardsiella tarda* and Gram-positive bacteria *Staphylococcus aureus.* However, recombinant *Le*-Hepc2 showed no activity against *Bacillus cereus*. Also r*Le*-Hepc2 was found to be non-haemolytic and non-cytotoxic even at a concentration of

10 μM. Taken together, these preliminary data indicate an important role for *Le*-hepc2 in the innate immunity of *Leignathus equulus* and suggest its potential application in aquaculture as therapeutics.

Keywords: Antimicrobial peptides; Hepcidin; *Leiognathus equulus*; Innate Immunity; Recombinant expression.

*These authors have contributed equally to this work.

[§]Corresponding author. Tel: + 914842368120, Fax: + 914842381120

E-mail address: rosammap@gmail.com