RECOMBINANT EXPRESSION AND FUNCTIONAL CHARACTERIZATION OF ANTIMICROBIAL PEPTIDE CRUSTIN FROM *ARTEMIA SALINA*

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ABSTRACT

Crustins are cationic Antimicrobial peptides (AMPs) containing a whey acidic protein (WAP) domain at the C-terminus. The present study is focused at recombinant production and functional characterization of crustin-I AMP isoform from the brine shrimp, *Artemia salina* (As-Crustin). As-Crustin sequence was highly similar to the crustins of *Scylla serrata* and *Scylla paramamosain* and shared 88 % identity among each other. The mature peptide of As-Crustin is composed of 90 amino acids with a molecular weight of 10 kDa, p*I* of 8.0 and net charge +3. The WAP domain was the only domain found in the As-Crustin sequence obtained in the present study. This domain forms a tightly packed structure described on PROSITE as a four-disulphide core (4DSC). Peptide models of As-crustin composed of a tightly coiled structure enclosing two β-sheets but no helices or possibly a loop protein. Active peptide of As-Crustin with WAP domain was produced by heterologous expression in *E. coli*, Rosettagami B DE3 pLysS using the pET-32a+ vector. The purified and refolded rAs-Crustin protein exhibited antimicrobial activity at a concentration of 2.5 μM against Gram-negative bacteria *Pseudomonas aeruginosa* and *Edwardsiella tarda* and Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus.* Recombinant peptide was not hemolytic or cytotoxic even at a concentration of 6 μM. Results from the functional studies propose that rAs-Crustin is a promising potent therapeutic agent against bacterial infection.

Keywords: Antimicrobial peptides; Crustin; *Artemia salina*; Innate Immunity; Recombinant protein.

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