

Cloning and expression of a Hemocyanin isolated from the centipede *Cryptops iheringi*

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INTRODUCTION

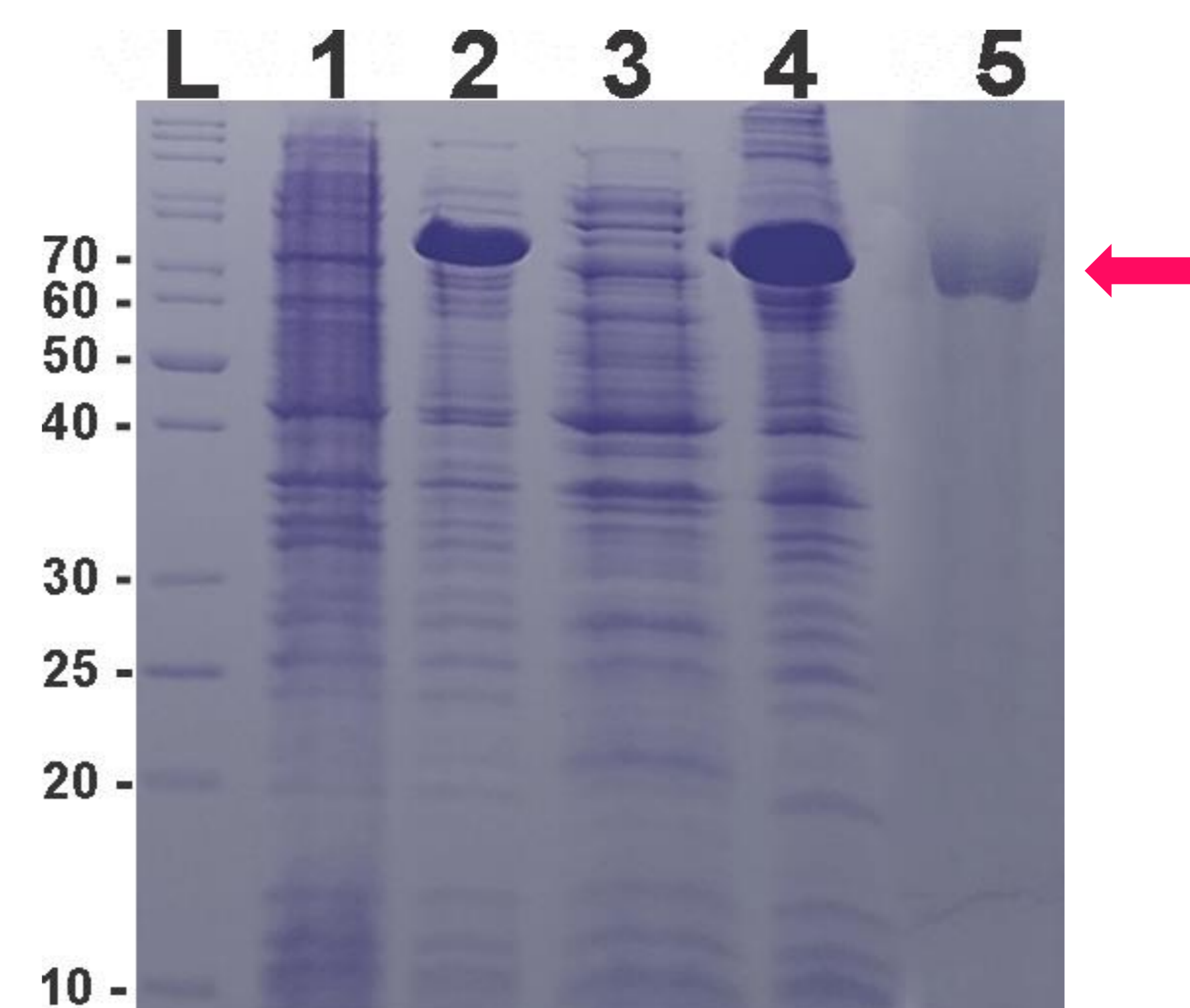
In this work a transcriptomic analysis of the *C. iheringi* venom gland was performed to obtain a profile of the toxins of this species. In addition, the crude venom was subjected to mass spectrometry analysis to establish an association between unknown sequences. These approaches for the construction of a general profile of the venom gland expression of this species led to the identification of a Hemocyanin (Hc) subunit.

Hemocyanins are copper-containing, respiratory proteins that occur in the haemolymph of many arthropod species. Here, we report the presence of Hc in the chilopode Myriapoda *C. iheringi*. Such respiratory proteins have long been considered unnecessary in Myriapoda, due to its tracheal systems.

These respiratory proteins are potent immunogens, which induce the synthesis of large amounts of specific antibodies. Studies pointed out its interaction with polymorphonuclear monocytes and lymphocytes and in vitro tests have shown a potential anticancer activity, with in vitro significant inhibition of the growth of cancerous strains of the breast, pancreas and prostate. Currently scientific data is mostly limited to the study of native Hc of *M. crenulata* molluscs, therefore, the biotechnological potential of Hcs isolated from centipedes is still unexplored.

RESULTS AND CONCLUSION

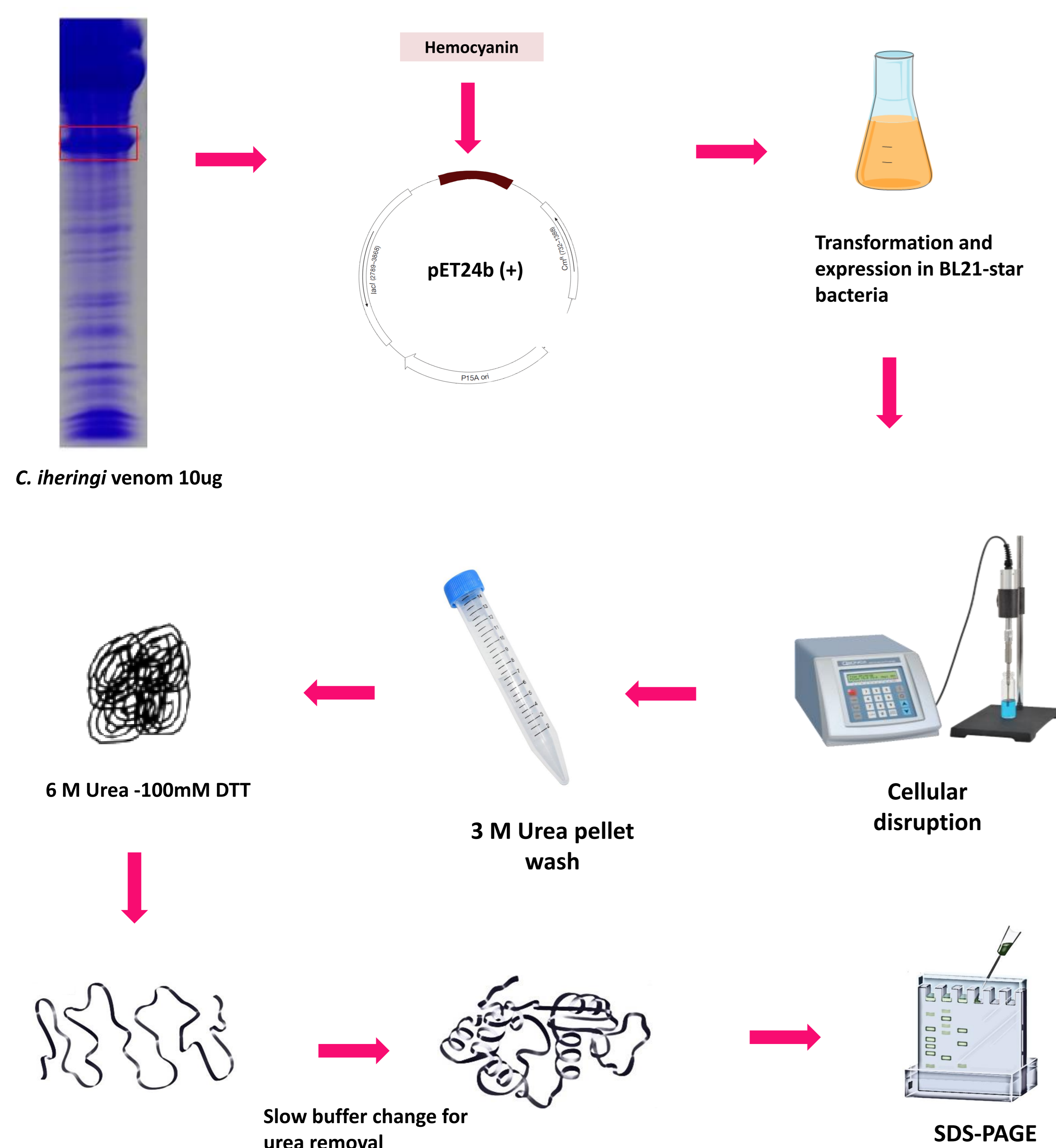
The Hc sequence have a 76 kDa range. The Hc subunit sequence was successfully expressed as inclusion bodies. Refolding attempts provided soluble forms of Hc that now will be used to explore its anticancer activity.



SDS-PAGE 12% containing the expression and refolding of Hemocyanin after 4h of 1mM IPTG induction. Stained with Coomassie blue. L- Ladder, 1- bacterial pellet before 1mM IPTG induction, 2 - bacterial pellet after IPTG induction, 3- supernatant, 4- Refolded protein.

METHODS

The Hc subunit sequence was synthesized with codon optimization for bacteria expression and the protein expressed as inclusion bodies. Refolding attempts provided soluble forms of Hc.



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THE AUTHORS ACKNOWLEDGE THE FINANCIAL SUPPORT OF



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