RECOMBINANT EXPRESSION OF TWO VARIANTS OF MICROPLUSIN (MPmH1A AND MPmH2A), AN ANTIMICROBIAL COPPER-CHELATING PEPTIDE.

Julia Lima¹, Iris de Araújo¹, Marcia A. Sperança¹, Luciano Puzer¹, Sirlei Daffre², Jose R. M. Pires³ e Fernanda Dias da Silva¹

¹Universidade Federal do ABC - Centro de Ciências Naturais e Humanas (CCNH); ²Universidade de São Paulo - ICB II - Depto. de Parasitologia; ³Universidade Federal do Rio Janeiro - Instituto de Bioquímica Médica

ABSTRACT
Microplusin is a Rhipicephalus (Boophilus) microplus anionic antimicrobial peptide, with six cysteine residues and with histidine-rich regions at the N and C termini. Its structure consists of a single α-helical globular domain, with five α-helices. It was demonstrated that microplusin binds copper II and iron II and the histidine H-1, H-2 and H-74 are among the possible amino acids involved in forming the binding site for copper. Studies revealed it is active against several Gram-positive bacteria and fungi. In fact, microplusin showed a bacteriostatic and fungistatic activity against Micrococcus luteus and Cryptococcus neoformans, respectively. This growth inhibitory effect is abrogated by copper supplementation and studies have suggested that the antimicrobial activity of microplusin is related to its copper-chelating property. Interestingly, the supplementation of culture media with iron II did not affect the microbiostatic effect of microplusin. It is probably that the copper binding by microplusin affects the activity of the copper dependent enzymes of microorganisms. In fact, microplusin inhibited respiration in Micrococcus luteus, probably by affecting the activity of heme-copper oxidases. Besides, it also inhibited the melanization of C. neoformans, a process dependent of laccase, an enzyme copper dependent. Here, we evaluated the effect of replacing of the amino-terminal histidines 1 and 2 (H-1 and H-2) to alanine residues on microplusin activities. For this, we cloned both variants (MPmH1A and MPmH2A) in pRSET A vector for expression in Escherichia coli BL-21. At the moment, purification phase is underway with significant results for the expression of the peptides. The next step comprises the assays against Micrococcus luteus to evaluate their antimicrobial action and the analysis of both MPmH1A and MPmH2A copper chelating activity by mass spectrometry. Key Words: Microplusin; antimicrobial peptide; copper-chelating. Acknowledgements: FAPESP.