

Antimicrobial factors from the harvestman *Acutisoma longipes*.

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Introduction: The arthropods defend themselves against invading microorganisms and parasites through the cellular and humoral immune systems. The cellular reactions are carried out by the blood cells (hemocytes) which immobilize the invaders by phagocytosis and/or encapsulation. The humoral response is performed by constitutive and inducible factors in the hemolymph. These molecules may be involved in recognition, facilitation of cellular immune response or direct antibacterial action.

Objectives: The objective of this study was to identify antibacterial factors in the harvestman's hemolymph, *Acutisoma longipes* (Gonyleptidae, Opiliones). Harvestmen are an interesting group of arachnids for the purposes of antimicrobial activity investigation because they are arachnids that feed on dead insects, plant juices and on substances in the process of decomposition. The characterization of antimicrobial peptides in this class of chelicerates would be of great value to understand the evolutionary aspects of arthropods' innate immunity. Harvestmen *Acutisoma longipes* are very common in grottos and caves and the ones object of the present investigation were collected in grottos around the Pedra Grande site, located in the City of Atibaia, State of São Paulo. **Methods:** The hemolymph (1.6 mL) was collected from prechilled animals by posterior legs articular membrane puncture using an apyrogenic syringe with buffer solution of citrate of sodium. The hemocytes were separate from the plasma by centrifugation, the plasma and the hemocytes were washed separately in buffer solution of citrate of sodium and submitted to acid extraction. The acid extract of the plasma and the hemocytes were submitted to a pre-purification in Sep-Pak C18 and then, such extract was eluted with different acetonitrile concentration (5%, 40% and 80% ACN) in trifluoroacetic acid (TFA) 0.046%. All the fractions were concentrated in a vacuum centrifuge, reconstituted in TFA 0.046% and loaded into a semi-preparative C18 Vydac column using a linear gradient of ACN in TFA 0.046% for the second purification step. The active fractions were then loaded into an analytical C18 Vydac column for a third purification step. The column effluent was monitored by absorbance at 225 nm and the antibacterial activity was determined by liquid growth inhibition assay. Active fractions from the analytical column have their purity and masses valued by mass spectrometry in a MALDI/TOF and ESI-MS devices. **Results and discussion:** Thirteen factors inhibited the growth of the Gram-positive bacteria *Micrococcus luteus* A270: eleven from the plasma and two from the hemocytes. One fraction from the plasma named P3a ($[M+H]^+ = 2,126$ Da) is pure, and its sequence of aminoacids is under analysis. Another fraction from the plasma named P2 showed the presence of two different molecules: one with $[M+H]^+ = 2,213$ Da, and another with $[M+H]^+ = 2,342$ Da. The low concentration of the molecules obtained from the hemolymph suggests that harvestmen, like insects, need to be challenged to increase the expression of antimicrobial peptides from the immune system. Therefore, the activity founded in unchallenged harvestmen seems to be basal.

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