

New Potential Antimicrobial Peptides Sequenced From *Acanthoscurria gomesiana* Venom By A Mass Spectrometry-Based Strategy

Thiago F. Abreu¹; Bianca N. Sumitomo¹; Milton Y. Nishiyama Junior²; Ursula C. de Oliveira²; Eduardo S. Kitano²; André Zelanis³; Solange M. T. Serrano²; Inácio Junqueira de Azevedo²; Pedro I. da Silva Jr²; Alexandre K. Tashima¹

¹Departamento de Bioquímica, UNIFESP, SP; ²Laboratório Especial de Toxinologia Aplicada, CeTICS - Instituto Butantan, SP; ³Departamento de Ciência e Tecnologia, ICT-UNIFESP, SP, Brazil.

INTRODUÇÃO. The Brazilian spider *Acanthoscurria gomesiana* inhabits regions of Southeastern Brazil and is mostly known by the potent antimicrobial peptides gomesin and acanthoscurrin, discovered from the spider hemolymph. However, its venom remains unexplored to date. **OBJETIVO:** In this study, we have performed a peptidomic analysis of *A. gomesiana* spider venom applying a MS-based workflow in order to completely sequence venom peptides and also to evaluate the antimicrobial activity of the peptide fraction against the Gram negative bacteria *Escherichia coli*. **MATERIAL E METODOS:** Venoms were extracted by electrical stimulator and stored at -80°C until use. Proteins were digested with four enzymes (trypsin, chymotrypsin, thermolysin and glu-C) for 15 minutes and both digested and intact peptides were submitted to separation in a capillary C18 column, followed by electrospray ionization and tandem mass spectrometry analyses applying data dependent and data independent LC-MS/MS acquisition methods. Digested peptides were analyzed by automated *de novo* sequencing, followed by searches against Araneae, *Acanthoscurria geniculata* and a custom *Acanthoscurria juruenicola* transcriptomic databases. All peptides were manually validated. **DISCUSSÃO E RESULTADOS:** MS analysis of the intact peptides has revealed that *A. gomesiana* venom peptidome fraction is composed by less than 165 features, with masses ranging from 0.4-15.8 kDa. The multiple enzymatic digestion peptides strategy analysis has identified 135 peptides belonging to 17 proteins, including three new completely sequenced mature peptides: U1-TRTX-Agm1a, U1-TRTX-Agm2a and U1-TRTX-Agm3a, which contains 3, 4 and 3 disulfide bonds, respectively. The first peptide, U1-TRTX-Agm1a, is homologous to U1-TRTX-Ap1a from *A. paulensis*, differing by only one amino acid, while U1-TRTX-Agm2a presents full similarity with genicutoxin-D1 mature peptide from *A. geniculata*. After preliminary antimicrobial activity assays of the peptide fraction of *A. gomesiana* venom against *Escherichia coli* resistant strains, we suggest that these toxins have potential applications as antimicrobial agents.. **CONCLUSÃO:** We have determined that *A. gomesiana* venom fraction presents three new peptides, which may be related to its antimicrobial activity.

Palavra chave: Peptidomics, *Acanthoscurria gomesiana*, Antimicrobial Activity
Patrocínio: FAPESP and CAPES