Transcriptomic and proteomic analyzes of the venom gland of Lasiodora subcanens and molecular characterization of fraction with cytotoxic activity

Dávila Regina Pacheco Silva[1], Frederico Francisco Fernandes[1], Camilo Elber Vital[1], Jaqueline Leal dos Santos[2], Thiago Geraldo Soares[2], Luana Silveira da Rocha Nowicki Varela[2], Sergio Verjovski-Almeida[3], Murilo Sena Amaral[3], Inácio de Loiola Meirelles Junqueira de Azevedo[3], Nancy da Ros[3], Pedro Marcus Pereira Vidigal[4], Rogerio Bertani[3], Márcia Helena Borges[2], William de Castro Borges[2]

[1.] UFOP, Universidade Federal de Ouro Preto, Ouro Preto / Minas Gerais / Brasil;
[2.] FUNED, Fundação Ezequiel Dias, Belo Horizonte / Minas Gerais / Brasil;
[3.] IB, Instituto Butantan, São Paulo / São Paulo / Brasil;
[4.] UFV, Universidade Federal de Viçosa, Viçosa / Minas Gerais / Brasil;

Spider venoms are compounds rich in biologically active molecules, which become targets for bioprospecting research, with relatively little explored biotechnological potential. The species Lasiodora subcanens is a Teraphoside not considered to be of medical relevance, so there is the possibility of obtaining its venom in a considerable volume. Some toxins present in the venoms of other species of the genus Lasiodora are described in the literature as having bioactivity, occurring, for example, in ion channels. Results of work previously developed by the author of this summary showed proteolytic and enzymatic activity of L. subcanens venom, as well as cytotoxic activity of its fractions against the Hs 578T tumor lineage (ATCC® HTB-126TM). The aim of the present work was to investigate the composition of L. subcanens venom, in addition to characterizing and sub-fractionating one of its fractions with cytotoxic activity, called P3, using proteomics and transcriptomics techniques. To achieve this, animal glands and poison were destroyed over the course of a year, forming pools. The venom glands were used to obtain mRNA, sequence the transcripts and, from the assembly and annotation of the transcriptome, the predicted proteins were used to create a database for the venom gland of L. subcanens. The extracted venom was fractionated in HPLC by molecular exclusion, to obtain the P3 fraction. The subfractionation of P3, by reversed phase, allowed obtaining the subfraction referring to the highest intensity peak by reading at 214 nm, called subfraction P3-7. The electrophoretic profile of the samples was then obtained and they were subjected to mass spectrometry, via MALDI-TOF and ESI-Orbitrap. The spectra obtained by ESI-Orbitrap were interrogated against the database assembled from the transcriptome. The proteins identified in subfraction P3-7 were modeled with the Swiss-model and PyMOL platforms, with stability analysis performed using the MolProbity tool. A P3 fraction presented m/z in the range of 1046-5700, while the P3-7 subfraction presented m/z in the range of 2836-5706, molecules with a net charge of +1, in accordance with that presented by SDS-PAGE. The crude venom presented 49 different proteins and 29 of them were identified in the P3 fraction; both samples contain proteins, enzymes, structural proteins, protease inhibitors, among others. While subfraction P3-7 contains 2 toxins and 1 CRISP (Cysteine-rich secretory protein). The main peptide of subfraction P3-7 presented 98.63% relative abundance (RA) and shares homology with a neurotoxin from Lasiodora parahybana. His model showed stability of 100% of its amino acids in permitted regions and 91.1% in favored regions. While the lowest abundance toxin model of P3-7, with 1.35% RA, showed amino acid stability of 100% in allowed regions and 97.1% for favored regions. Finally, this work opens perspectives for new cytotoxicity assays with the toxin of interest, most abundant in the P3-7 subfraction, seeking its mechanism of action and its effects on target cells. Furthermore, the curation of the transcriptome, in parallel with the use of new proteomic approaches, as well as the analysis of other fractions of the venom, may reveal new constituents of biotechnological interest, with different applicability, in addition to greater elucidation of its protein constitution.

Agradecimentos: CAPES - This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001. CNPq.

Fapemig. UFOP. Funed. Butantan. UFV.