Trypanosoma cruzi Nuclear Analysis: A Proteomic-Based Approach

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Trypanosoma cruzi, a flagellate protozoan, is the etiological agent of Chagas disease. During its life cycle, it differentiates into four main life stages in the both insect vector and mammalian host. *T. cruzi* proteomics have been performed using different proteomic strategies although no study on its nuclear subproteome has been reported. Epimastigote cells were subjected to mechanical lysis and subcellular fractionation by centrifugation on sucrose gradients. Enrichment of nuclear fraction was confirmed by western blotting. Nuclear proteins were separated by 2-DE. IEF for the 3-10 and 4-7 pH ranges was carried out using on the Ettan IPGphor3 (GE Healthcare) using the following steps:1h at 500 V, 1h at 750 V, 2:06 h at 10,000 V, 1h at 10,000 V. For 6-11 pH range, IEF was performed on MultiphorII (GE). The sample was applied near the cathode using the “paper bridge” method under the conditions: 1h at 150 V, 2 h at 300 V, 1h at 600 V, 10:30 h at 3,500 V. The resulting silver stained 2-DE gels were analyzed using the Image Master 6.0 Platinum software. 2-DE gels were also stained with colloidal CBB G 250, and spots subjected to PMF. Among the identified proteins were: tubulins, elongation factors, ATPases and several unknown proteins.

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