NARROW RANGE PH GRADIENT 2-DE MAPS OF *TRYPANOSOMA CRUZI* LIFE STAGES

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Trypanosoma cruzi is the etiological agent of Chagas disease. During its life cycle the parasite differentiates into the life stages epimastigotes, trypomastigotes and amastigotes. Here, we optimized protocols for 2-DE of *T. cruzi* proteins in the narrow pH ranges: 3-5.6 (Non-linear), 5.3-6.5 and 6.2-7.5. In all pH ranges used the gels were rehydrated with 360 µL of 7M urea, 2M tiourea, 65 Mm DTT, 2% Triton X100, 1% Pharmalite and the samples (0.4×10^8 cells/gel) applied on the anode using the paper bridge method. The IEF was carried out using the Ethan IPGphor III (GE Healthcare) following the protocols: a) 3-5.6: 1h 500 volts, 1h gradient 1000 volts, 3hs gradient 8000 volts, 2:40 hs 8000 volts; b) 5.3-6.5: 2hs 500 volts, 2 hs gradient 1000 volts, 3hs gradient 8000 volts, 7:40 hs 8000 volts and c) 6.2-7.5: 2hs 500 volts, 2hs gradient 1000 volts, 3 hs gradient 10000 volts, 9 hs 10000 volts. Among the gels the 3-5.6 pH range concentrated the majority of the spots, while the 6.2-7.5 pH gels displayed the lowest amount of spots. We are currently performing comparative image analysis of the gels in order to find differentially expressed proteins in T. cruzi life stages.
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