Proteomic Analysis of the *Leishmania major* Overexpressing the Nucleoside Diphosphate Kinase (LmNDK)

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Introduction: Leishmaniasis is an infectious disease caused by the protozoan parasite *Leishmania*, affects 14 million people in 88 countries and threatens 350 million people worldwide. The nucleoside diphosphate kinase (NDK) is ubiquitous in both prokaryotes and eukaryotes catalyzing the transfer of the $\gamma$-phosphoryl group from a NTP to a NDP by a ping-pong mechanism. Studies have demonstrated that NDKb is secreted by intracellular pathogens during infection, including by *Leishmania*, preventing ATP-induced cytolysis of macrophages, thereby preserving the integrity of host cells to the benefit of the parasite. We have recently shown that overexpression of the LmNDK led to a partial virulence attenuation in the BALB/c footpad lesion.

Objectives: In this work, we present a comparative analysis of the protein profile off the parasites using the promastigote *L. major* overexpressing the LmNDK.

Materials and Methods: The *L. major* promastigotes containing pX63NEO and pX63NEO-NDK were disrupted by osmotic lysis and the soluble fractions were precipitated using trichloroacetic acid. The gel strips were rehydrated and taken to isoelectric focusing. The second dimension gels were stained with Coomassie blue and captured images were analyzed using appropriate software.

Results and Discussion: The 2D-gels of the samples pX63NEO and pX63NEO-NDK showed about 260 spots with an overlap of 85%. Several spots of the gels were selected for MALDI-TOF analysis and identified using NCBI database. The 2D-gel analysis and the spot identifications showed an increased intensity of the LmNDK spot, confirming the protein overexpression.

Conclusions: The results showed that the protein extraction and production of two-dimensional gels protocols were efficient and allowed the analysis of protein expression in the LmNDK overexpressor. The protein identification of the differentially expressed spots may contribute to elucidate the LmNDK overexpression effects and the functions of this protein in the host-parasite interaction.

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