EXPRESSION OF THE RECOMBINANT TSA-1 ANTIGEN OF *TRYPANOSOME CRUZI* IN *ESCHERICHIA COLI*

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Introduction. Chagas disease caused by *Trypanosoma cruzi* affects approximately 10 million people; most of them live in poverty. The two drugs available for the treatment of this infection lack of efficacy and are expensive (1). Recent immunizations with DNA vaccines in animal experimental models demonstrated that TSA-1 is a potential antigen to develop a therapeutic vaccine using the properly folded recombinant protein (1, 2). *E. coli* is still the first choice for heterologus expression of proteins, and several *E. coli* strains are available to express recombinant proteins in a soluble and correctly folded conformation (3).
The aim of this work was to express the rTSA-1 antigen of *T. cruzi* in *E. coli,* as a soluble recombinant protein.

Methods. *E. coli* chemically competent cells of strains: BL21(DE3), C41, C43, pLemo21, Rosetta, and Rosetta 2 were transformed with the construct pET41A-TSA1. The bacteria were cultured in LB medium in the presence of the appropriate antibiotic at 37°C to a density of OD_{600}=0.6. Then, 1 mM isopropyl-ß-D- thiogalacto-pyranoside (IPTG) was added to induce the expression of rTSA-1 at 30 °C for 16 h. In the case of pLemo21, the expression of rTSA-1 was evaluated with different concentrations of L-rhamnose (4). The presence of rTSA-1 in total protein, soluble and insoluble fractions, was determined by SDS-PAGE and Western blot analysis using Anti-His commercial antibody (Invitrogen).

Results. Recombinant TSA-1 was expressed as inclusion bodies in all *E. coli* strains with the expected molecular weight. Interestingly, the rTSA-1 was detected in the soluble fraction in four out of six strain tested but with very low yield (Table 1). Then, the soluble expression of rTSA-1 in pLemo21 was improved by the addition of L-rhamnose up to 2000 µM (Fig. 1, lane 7). The yield of soluble rTSA-1 clearly increased, but it is still insufficient for a vaccine development. Therefore, the soluble expression should be further improved, or a refolding system has to inclusion bodies.

Table 1. Expression of rTSA-1 antigen in *E. coli*

Strain	Soluble fraction	Insoluble fraction
BL21(DE3)	ND	++++
pLemo(21)	++	++++
C41	+	++++
C43	ND	++++
Rosetta	+	++++
Rosetta 2	+	++++

Good ++, Poor +, No detected ND

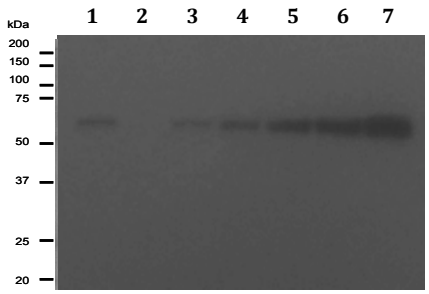

Fig.1 Expression of soluble rTSA-1 in *E. coli* pLemo21. 0 µM (control, lane 1), 100 µM (lane 2), 250 µM (lane 3), 500 µM (lane 4), 750 µM (lane 5), 1000 µM (lane 6), 2000 µM (lane 7) of L- rhamnose.

Conclusions. The rTSA-1 antigen of *T. cruzi* can be expressed in *E. coli* as a soluble recombinant protein.

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