EXPRESSION OF A LEGUMAIN FROM *TRICHOMONAS VAGINALIS* IN *ESCHERICHIA COLI* AND *PICHIA PASTORIS*.

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Introduction. *Trichomonas vaginalis*, a sexually transmitted parasite, has many cysteine proteinases (CPs); some are involved in trichomonal pathogenesis threfore, antibodies against some CPs have been detected in patient sera. The *tvlegu-1* gene from *T. vaginalis* encodes for a ~42.8 kDa precursor cysteine proteinase of the legumain type, dubbed TvLEGU-1(1). This CP is a virulence factor that plays a major role in trichomonal cytoadherence, and it is a potential biomarker for serodiagnosis of trichomonosis (2, 3). However, TvLEGU-1 *in vitro* characterization has been limited by the lack of its reliable recombinant expression in a correctly folded conformation.

Thus, the aim of this work was to obtain the recombinant precursor of TvLEGU-1 in a properly folded conformation.

Methods. 1.2 kb *tvlegu-1* gene was cloned into the expression vectors: pET-21b(+) (Novagene) and pGEX-6P-1(GE Healthcare) for the expression in *E. coli* and the pPICZαB (Invitrogen) for the expression in *P. pastoris*. For the expression in *E. coli,* chemically competent cells were transformed with the plasmid pET-21b(+)-*tvlegu1* or pGEX-6P-1-*tvlegu1*. The expression of TvLEGU-1 was induced with 0.5 mM IPTG at 37℃ during 3h. The expression of the TvLEGU-1 in *P. pastoris* was carried out as the Easy Select *Pichia* Expression Kit manual recommends (4). The expression and purification of TvLEGU-1 was followed by SDS-PAGE and WB assays using anti-TvLEGU-1 polyclonal antibody (3). In both cases TvLEGU-1 was purified by Ni-affinity chromatography.

Results. A summary of the recombinant expression of the two constructs of TvLEGU-1 in four different *E. coli* strains is shown in Table 1. TvLEGU-1 was expressed mainly as inclusion bodies (IB). It was also detected only in the soluble fraction in two strains; however the protein bands, recognized by the anti-TvLEGU-1 polyclonal antibody, have molecular weights lower than 42 kDa, suggesting that the precursor of TvLEGU-1 was expressed correctly folded, and probably its activation causes its auto digestion, as previously observed with other CPs (5). The recombinant protein expressed in *P. pastoris* was purified from the culture media as a soluble protein of 75 kDa, suggesting that it is properly folded. Moreover, this protein was recognized by anti-TvLEGU-1 polyclonal antibodies, indicating that it corresponds to the TvLEGU-1 precursor (Fig. 1). Further studies are needed to determine if the increase of the molecular weight is due only to the hyperglycosilation of the recombinant protein by *P. pastoris*.

Table. 1. Expresion of TvLEGU-1 in *E. coli*.

Plasmid	Strain	Expression
pET-21b(+)	BL21(DE3)	IB
	Shuffle	IB
	C43(DE3)/pREP4	Soluble*
	C43(DE3)/pREP4	Soluble*
pGEX-6P-1	BL21(DE3)	IB
	SHuffle	IB
	C43(DE3)	IB
	C41(DE3)	IB

*Several protein fragments were detected by WB assays

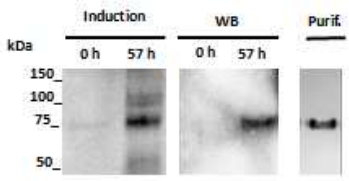

Fig. 1. Expresion of TvLEGU-1 in *P. pastoris*..

Conclusions. The TvLEGU-1 of *T. vaginalis* is expressed in *P. pastoris* as soluble and properly folded protein.

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References.

1. León-Félix J. Ortega- Lopéz J. Orozco-Solis R. and Arroyo R. 2004.*Gene*. 335: 25-35**.**
2. Ramón-Luing L. Rendón-Gandarilla F. Cárdenas-Guerra R. Rodríguez-Cabrera N. Ortega-López J. Avila-González L. Angel-Ortiz C. Herrera-Sánchez C. Mendoza-García M. Arroyo R. 2010. *Proteomics.* 10: 435-444
3. Rendón-Gandarilla F. Ramón-Luing L. Ortega-López J. Rosa de Andrade I. Benchimol M. Arroyo R. 2012. *J. Biomed. and Biotech*. 2013.
4. Easy Select *Pichia* Expression Kit: User Manual. Invitrogen.
5. Ocádiz R, Orozco E, Carrillo E, Quintas LI, Ortega-López J, García-Pérez RM, Sánchez T, Castillo-Juárez BA, García-Rivera G, Rodríguez MA. 2005. *Cell Microbiol*. 7(2):221-32.