EXPRESSION OF THE CATALYTIC DOMAIN OF A CYSTEINE PROTEINASE FROM *TRICHOMONAS VAGINALIS* IN *E. COLI* AS A SOLUBLE POLYPEPTIDE IS NOT SUFFICIENT TO HAVE ENZYME ACTIVITY

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Introduction. *Trichomonas vaginalis* encodes multiple proteinases mainly of the cysteine type (CPs) (1). Several, trichomonad CPs are virulence factors that play an important role in the pathogenicity of the parasite (2). TvCP4 is a CP of *T. vaginalis* up-regulated by iron at the posttranscriptional level by an IRE/IRP-like system (3). It is also potential biomarkers for trichomonosis (4). However, the biochemical characterization of TvCP4 has been limited, manly by the lack of a reliable source of active enzyme. CPs are synthesized as inactive precursors (proenzyme) that have a pre-pro region (prodomain) and a catalytic domain (mature enzyme). Changes in pH and/or Red-Ox environment trigger the digestion of the pre-pro region, mainly in an auto processing step, leading to the activation of CPs (5). Efforts to obtain TvCP4 as an active enzyme by the activation of its refolded precursor have failed.

The aim of this study was to determine whether the TvCP4 proteolytic activity can be recovered from its soluble recombinant catalytic domain (TvCP4m).

Methods. Culture of *E. coli* BL-21 (DE3) strain harboring the TvCP4 catalytic domain construct were grown at 37°C and 200 rpm in different grow media (TB, LB, MZY and 2TY) and expression of the recombinant protein was induced by the addition of IPTG (0.5 mM) at OD600= 0.6. at 37°C for 5 h. Then, to improve the expression of TvCP4m in the soluble fraction three *E. coli* strains (Rosetta-gami™, C43, and C41) were tested. Presence of TvCP4m in the soluble fraction was checked by SDS–PAGE using 10% polyacrylamide gels and Western Blot assays using an anti-TvCP4m antibody.

Results. The expression of the catalytic domain of TvCP4 in all the *E. coli* strains and growth conditions tested was mainly as inclusion bodies. Interestingly, assessment of the TvCP4m expression on different strains shows that Rosetta-gami™ and C41 strains achieved an appreciable amount of soluble TvCP4m (Fig 1). To improve the expression of soluble TvCP4m, Rosetta-gami™ and C41 were grown in different media. However, only a slight increase of soluble TvCP4m was obtained in TB medium, but no enzyme activity was observed in the zymograms for neither the soluble or insoluble fraction. These results suggest that in the case of this CP the expression of a soluble polypeptide is not sufficient to achieve an active conformation.

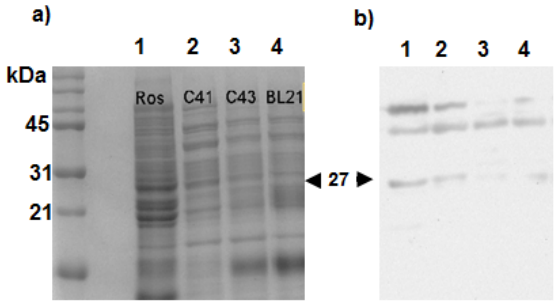

Fig.1. Expression of TvCP4m in soluble fraction of different *E. coli* strains. Panel a) SDS-PAGE gel. b) Western Blot assay. Lane 1: Rosetta-gami™, lane 2: C41, lane 3: C43, and lane 4: BL21.

Conclusions. The concentration of soluble TvCP4m was expressed in *E. coli* strains Rosetta-gami™ and C41 as a soluble polypeptide, but without enzyme activity.

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