The Cysteine Synthase enzyme plays an important role in the biological cycle of *Trypanosoma cruzi.*

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Introduction

Trypanosoma cruzi can use amino acids as energy sources and to support several biological processes such as differentiation, resistance to stress conditions and host-cell invasion[1]. Cysteine is a sulfured amino acid relevant for the maintenance of redox homeostasis, and has been associated with cellular defense mechanisms against oxidative imbalance[1]. The de novo cysteine biosynthesis pathway is comprised of serine O-acetyltransferase (SAT) and cysteine synthase (CS) enzymes which sequentially mediate two consecutive steps of cysteine biosynthesis[2].

Results

Cloning, expression, purification and structure of recombinant TcCS

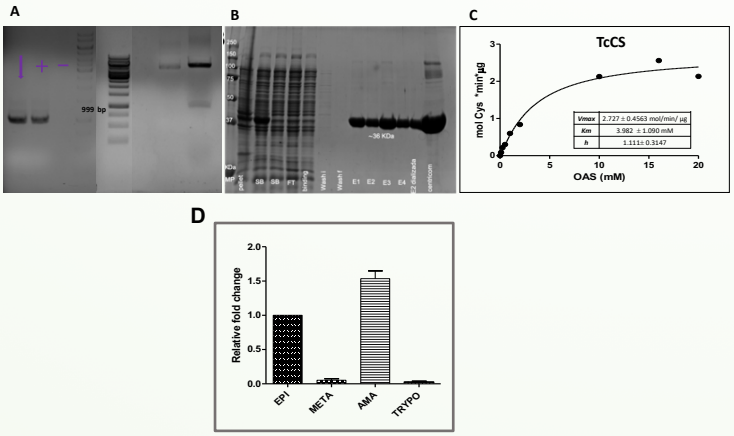

Figure 1: Cloning, expression, purification, enzyme activity and structure TcCS. **A**. Cloning and restriction analysis. **B.**Purification of TcCS by Ni $^{2+}$ - NTA. **D**. TcCS enzyme activity: L-Cys production at 560 nm was measured using a spectrophotometer (Thermo, UV visible-evolution 300) with the method described by Gatonde (1967). The data was analyzed using the Hill equation. **D**. Relative levels expression for TcCS in the diferente stages of life cylce by RT-PCR

Partial knockout mutants for *TcCS* obtained by CRISPR-Cas9

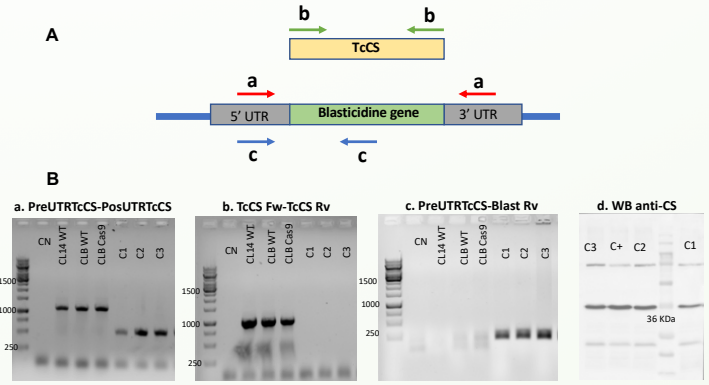

Figure 2. Knockout parasites by CRISPR-Cas9. Epimastigotes of *T. cruzi* CL Brener strain, expressing constitutive pLEW13/Cas9, were transfected with guide RNA and DNA donor. **A.** Experimental scheme for exchanging the gene of interest for a gene that confers resistance. **B.** We obtained three TcCS clones verified by PCR using three pairs of oligonucleotides: **a**. Oligonucleotide amplification of UTR regions of TcCS (1350 bp), **b**. Oligonucleotide for ORF of TcCS (999 bp), **c**. Oligonucleotide in the preUTR region of TcCS and another region Blasticidine resistance gene (210 bp), and **d**. Evaluation of protein content by WB with specific anti-TcCS antibody.

The partial *ΔTcCS* knockouts tigger different phenotypes during the life cycle

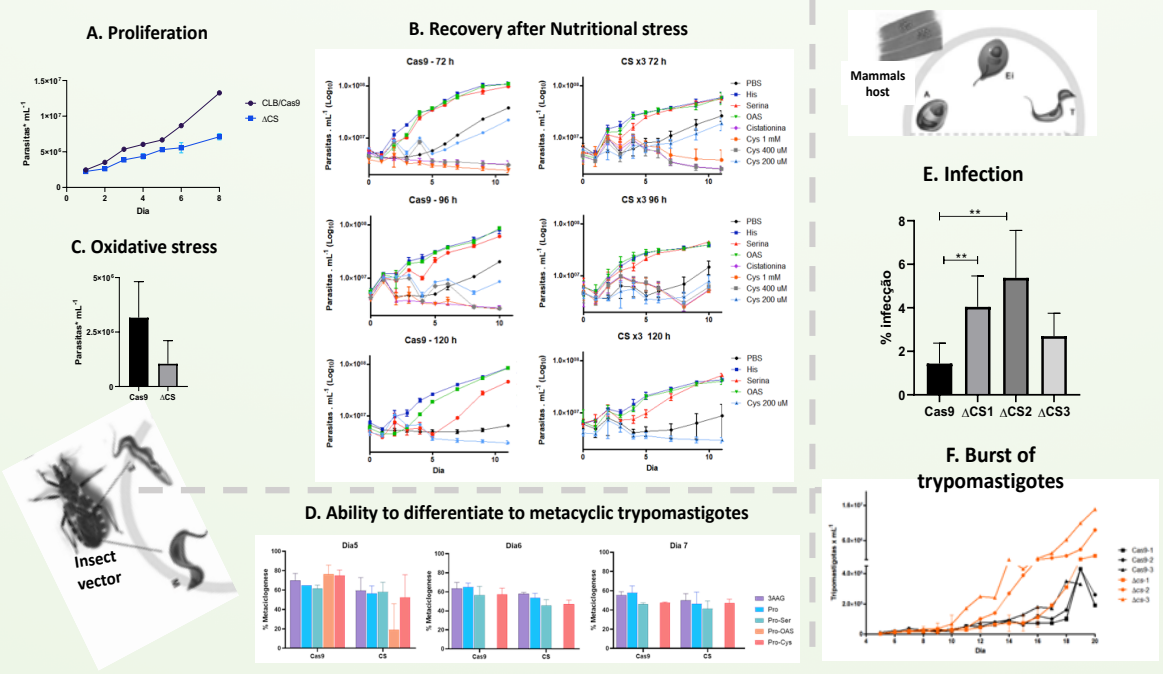

Figure 3.Phenotype in ΔTcCS partial knockout parasites by CRISPR-Cas9. Insect vector: **A**. Epimastigote forms with lower proliferation rates, **B**. These parasites were submitted to nutritional stress in the presence (or not as a control) of 5 mM L-Serine, 5 mM OAS or 0.2, 0.4 or 1 mM L-Cys we observed that: i. L-Cys concentrations over of 200 µM were lethal to the mutants after 48 hours and; ii. L-Ser and OAS contributed to the survival of both, mutants and wild type parasites to severe starvation, and **C**. They are resistant to 120 µM to H_2O_2 during 30 min of exposition; **D**. We found that ΔTcCS parasites had diminished their ability to differentiate to metacyclic trypomastigotes. Mammals host. **E**. When these metacyclic trypomastigotes were evaluated for their ability to infect mammalian host cells, we observed an over rate of infection after 48 hours, and **F.** an increment of trypomastigote bursting with some morphological differences when compared to the control.
E: Epimastigotes, M: Trypomastigotes Metacyclics, A: Amastigotes and T: Trypomastigotes

Conclusion and perspectives

TcCS (PLP-dependent enzyme) was expressed as soluble and active enzyme. We selected knockout mutants obtained by the CRISPR-Cas9 methodology for ΔTcCS-Blast. The phenotype data indicate that cysteine has an important role during epimastigotes proliferation, metacyclogenesis and the infection of mammalian cells, which could prompt this enzyme as a possible drug target. Further experiments will allow us to better understand the role of the cysteine biosynthesis *de novo* for the biology of *T. cruzi.*

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References

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