Why not use His? Generating of *Trypanosoma brucei* cell lines expressing *Trypanosoma cruzi*'s histidine degradation pathway

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INTRODUCTION

Histidine (His) is an amino acid highly abundant in triatomine's gut and that can be used by *Trypanosoma cruzi* as an ATP source (Barisón et al., 2016). The histidine (His) degradation pathway has four putative enzymatic steps in *T. cruzi*. However, this pathway is absent in other trypanosomatids, such as *Trypanosoma brucei*, what makes this organism a great model for the study of the His degradation pathway.

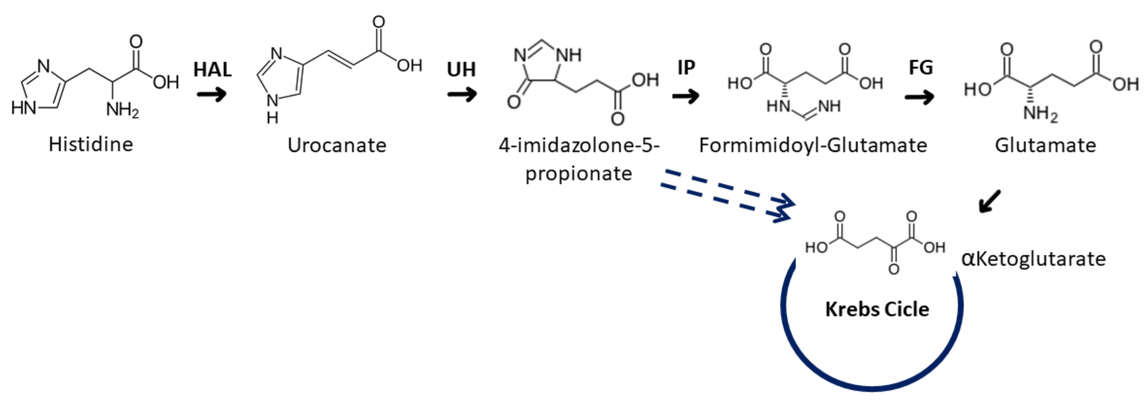

Figure 1. His degradation pathway present in *T. cruzi*. HAL: histidine ammonia lyase, UH: urocanate hydratase, IP: imidazolonepropionase, FG: formimidoylglutamase.

OBJECTIVES

The main question of this work is:

What is the evolutionary advantage that this pathway brings to this parasite?

To answer this question, the specific goals of this work are:

- To generate *T. brucei* cell lines that express the His degradation pathway, both partially and complete;
- To phenotypically analise those cell lines by evaluating their capacity to:
  - uptake His
  - oxidize His to CO_2
  - resist severe nutritional stress.

METHODS

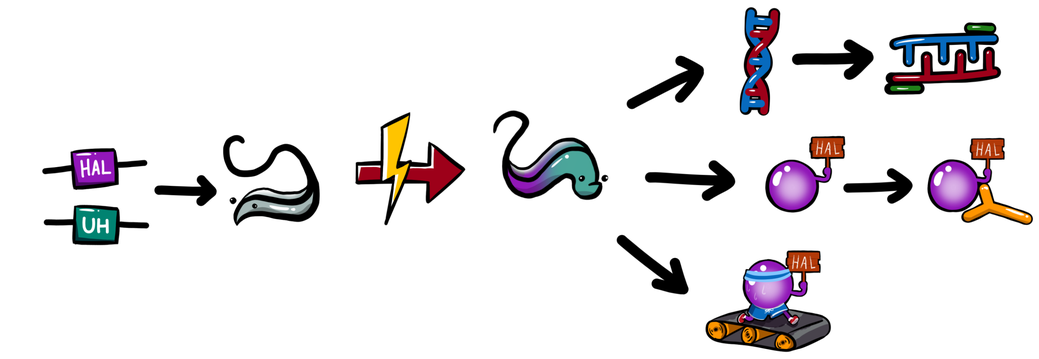

Figure 2. Methods – generation of mutant cell lines. Parasites were transfected by electroporation with linearized plasmids containing the codifying sequences of TcHAL and TcUH. Clones were selected by limited dilution and antibiotics. Polymerase Chain Reaction (PCR) was used to confirm the insertion of the codifying sequences in the selected clones gDNA, western blotting analysis was performed to confirm enzymes expression and enzymatic activity was measured to confirm both enzymes were expressed in their active form.

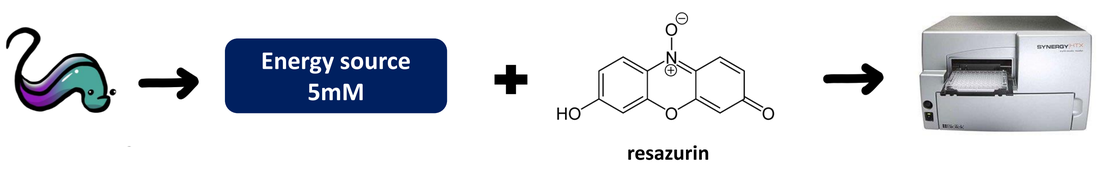

Figure 3. Methods – cell viability under nutritional stress. Parasites were washed and incubated in PBS buffer without any energy source (negative control) and with glucose, proline, histidine (His) and urocanate (Uro). Then, cells were incubated with resazurin for two hours and cell viability was measured by fluorescence using fluorimeter.

RESULTS

T. brucei procyclic cell line HAL+UH expresses both TcHAL and TcUH constitutively

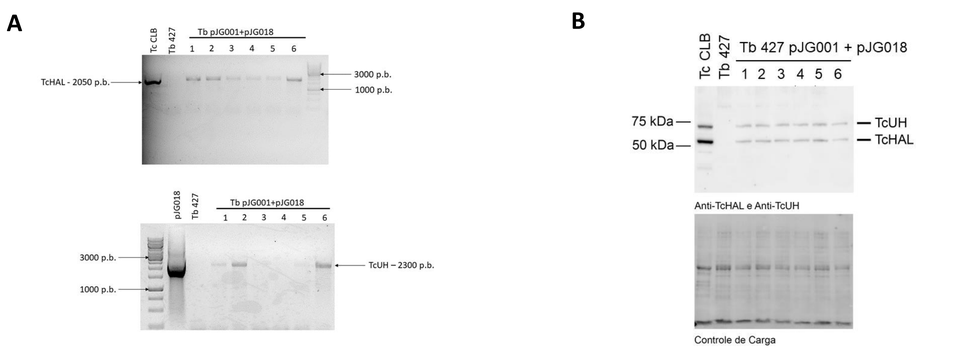

Figure 4. A. TcHAL and TcUH codifying sequences are inserted in the gDNA of procyclic T. brucei HAL+UH cell lines. Polimerase chain reaction using specific primers for each enzyme. **B. Clones from procyclic *T. brucei* pJG001+pJG018 cell line express TcHAL and TcUH.** Western blotting using total cell extracts from pJG001+pJG018 and controls probed with anti-TcHAL antibody (1:1000) and anti-TcUH antibody (1:400). TcCLB – Positive control – wild-type *T.cruzi* CLBrener strain Tb427 – Negative control – wild-type *T. brucei* 427 strain; Tb pJG001+pJG018 cl1 to cl6 – pJG001+pJG018 transfected procyclic *T. brucei* 427 parasites, expressing TcHAL and TcUH.

RESULTS

T. brucei procyclic cell line HAL+UH expresses TcHAL and TcUH in their active form but the ratio of expression differs from *T. cruzi*

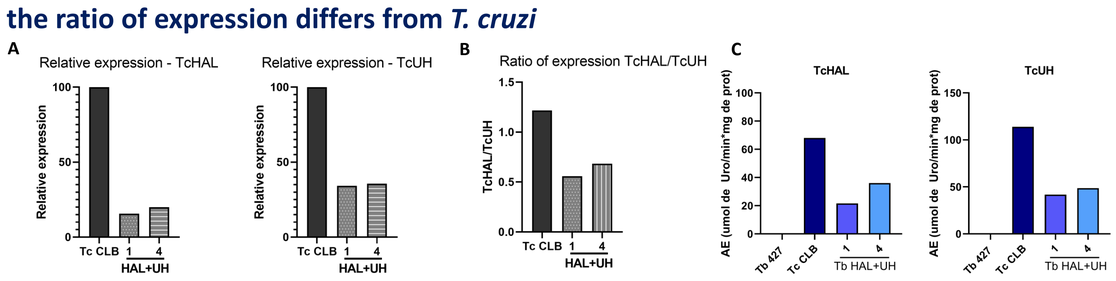

Figure 5. A. Clones from *T. brucei* pJG001+pJG018 cell line express less TcHAL and TcUH than epimastigotes *T. cruzi* CLB cell lines. Western blotting quantification using ImageJ software. **B. Enzyme expression is different in clones when compared with control.** Ratio of expression TcHAL/TcUH. **C. *T. brucei* pJG001+pJG018 cell lines express TcHAL and TcUH in its active form.** Enzymatic activity assay using protein extracts from transfected and control cell lines. TcCLB – Positive control – wild-type *T.cruzi* CLBrener strain; Tb427 – Negative control – wild-type *T. brucei* 427 strain; Tb HAL+UH cl1 and cl4 – pJG001+pJG018 transfected procyclic *T. brucei* 427 parasites, expressing TcHAL and TcUH

Expression of TcHAL and TcUH simultaneosly affects parasite proliferation

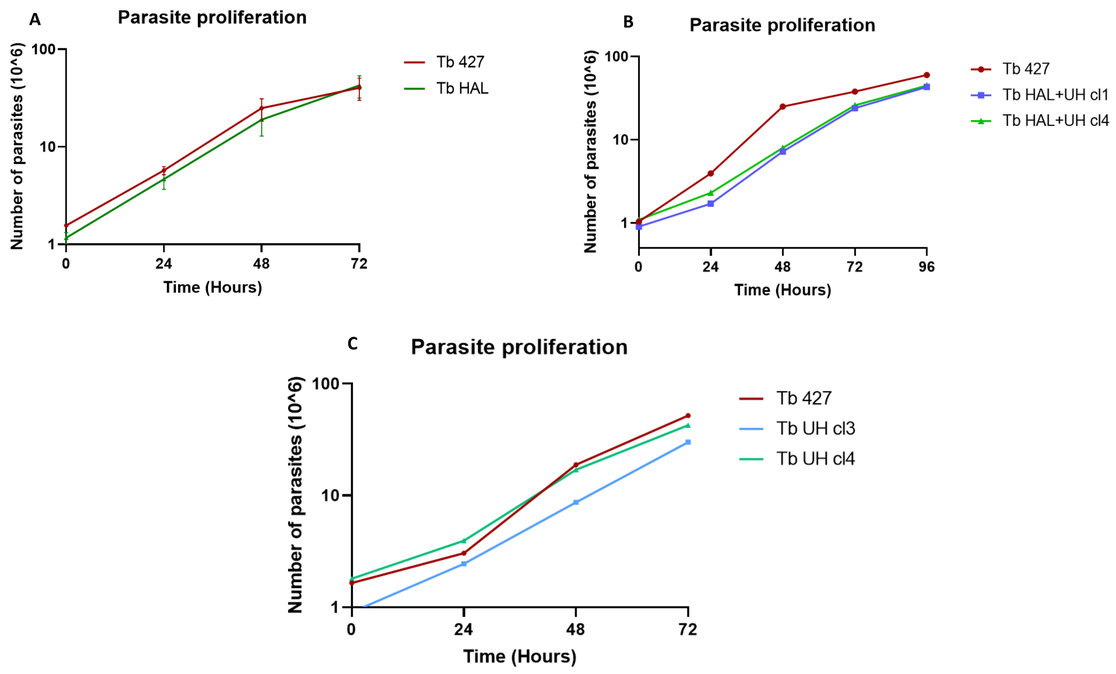

Figure 6. Procyclic *T. brucei* cell lines expressing either TcHAL (A) or TcUH (B) proliferate similarly to *T. brucei* wild type cell lines. C. Expression of both enzymes affects parasite proliferation of *T. brucei* HAL+UH cell lines compared to wild type. Parasites were incubated 72-96 hours in rich medium and counted in Neubauer chamber. Tb427 – Negative control – wild-type *T. brucei* 427 strain; Tb HAL+UH cl1 and cl4 – pJG001+pJG018 transfected procyclic *T. brucei* 427 parasites, expressing TcHAL and TcUH

Urocanate diminishes cell viability faster than the negative control in *T. brucei* HAL+UH cell lines submitted to nutritional stress

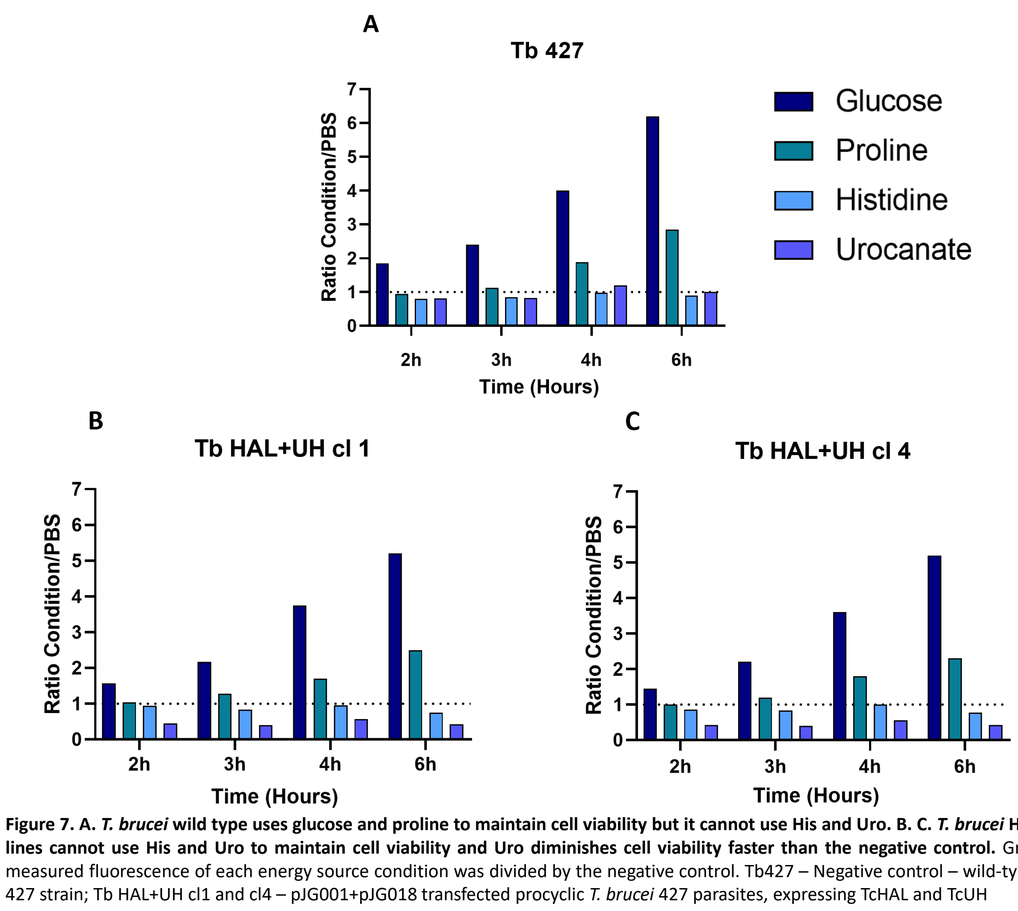

Figure 7. A. *T. brucei* wild type uses glucose and proline to maintain cell viability but it cannot use His and Uro. B. C. *T. brucei* HAL+UH cell lines cannot use His and Uro to maintain cell viability and Uro diminishes cell viability faster than the negative control. Graphs show measured fluorescence of each energy source condition was divided by the negative control. Tb427 – Negative control – wild-type *T. brucei* 427 strain; Tb HAL+UH cl1 and cl4 – pJG001+pJG018 transfected procyclic *T. brucei* 427 parasites, expressing TcHAL and TcUH

CONCLUSIONS

- *T. brucei* cell line HAL+UH constitutively express TcHAL and TcUH simultaneously and the expression of these enzymes affects parasite proliferation;
- *T. brucei* cell line HAL+UH can use both enzymes to catabolize His, but with reduced specific activity when compared to *T. cruzi* epimastigotes.
- Urocanate diminishes cell viability faster than the negative control in *T. brucei* cell lines HAL+UH submitted to nutritional stress

REFERENCES

BARISÓN, M. J.; DAMASCENO, F. S.; MANTILLA, B. S.; SILBER, A. M. The active transport of Histidine and its role in ATP production in *Trypanosoma cruzi*. **Journal of Bioenergetics and Biomembranes**, v. 48, n. 4, p. 437–449, 2016.