Deepening the knowledge of the branched chain amino acids catabolic pathway in *Trypanosoma cruzi:* the role of an Enoyl-CoA hydratase

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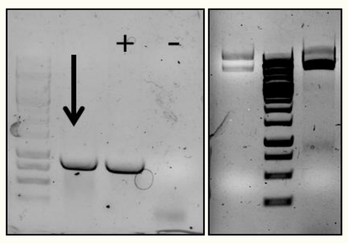
Introduction

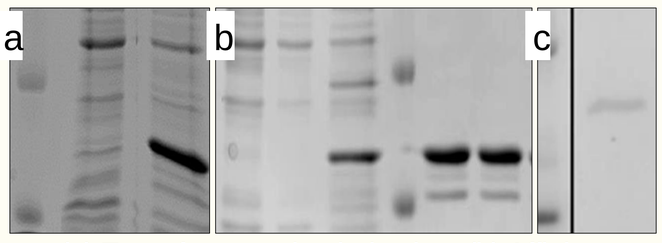
Enoyl-CoA hydratase (ECH), or crotonase, catalyzes the second step of the β-oxidation pathway and has also been implicated in the fourth step of the branched-chain amino acids (BCAA - Leu, Val, Ile) catabolic pathway. We are interested in describing how *T. cruzi* metabolizes BCAA and the contribution of the components of this metabolic pathway to the development of the parasite's life cycle. In *T. cruzi*, the branched-chain keto acids derived from BCAA are further oxidized by the α-keto acid dehydrogenase complex and dehydrogenated by acyl-CoA dehydrogenases[1]. Subsequently, the products can be metabolized by an ECH (TcECH), producing acetyl-CoA which in turn can feed the tricarboxylic acid cycle. With this work we want contribute to a better understanding of the participation of TcECH in the BCAA catabolism. The biological implications of TcECH for the life cycle allow us to suggest it as a new target for treatment against this parasite.

1. Cloning, expression, purification and biochemical analysis of recombinant TcECH

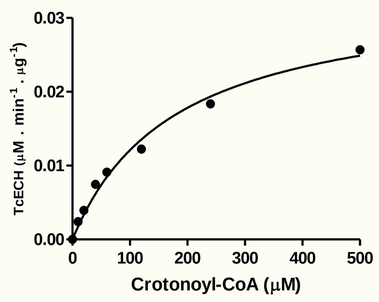
Gene: TcCLB.508153.130



PCR and restriction analysis

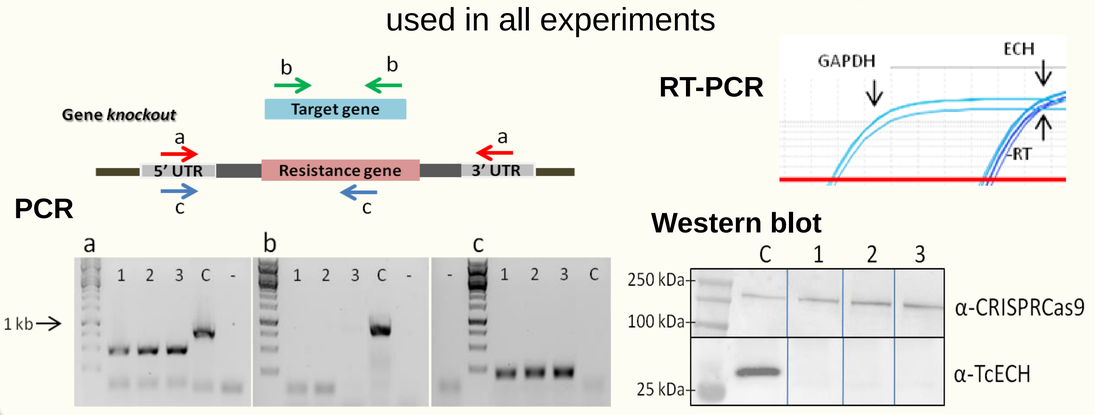


(a) Test of expression induction. (b) Affinity purification process. (c) Western blot using α-6xHisTag antibody



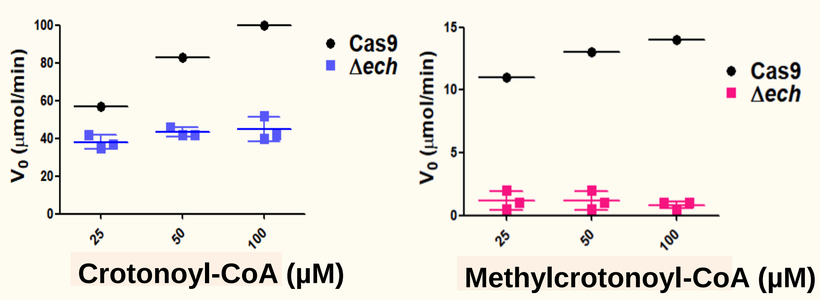
2. TcECH knockout parasites obtained by CRISPR-Cas9

Three independent clones of the knockout cell line were generated and used in all experiments

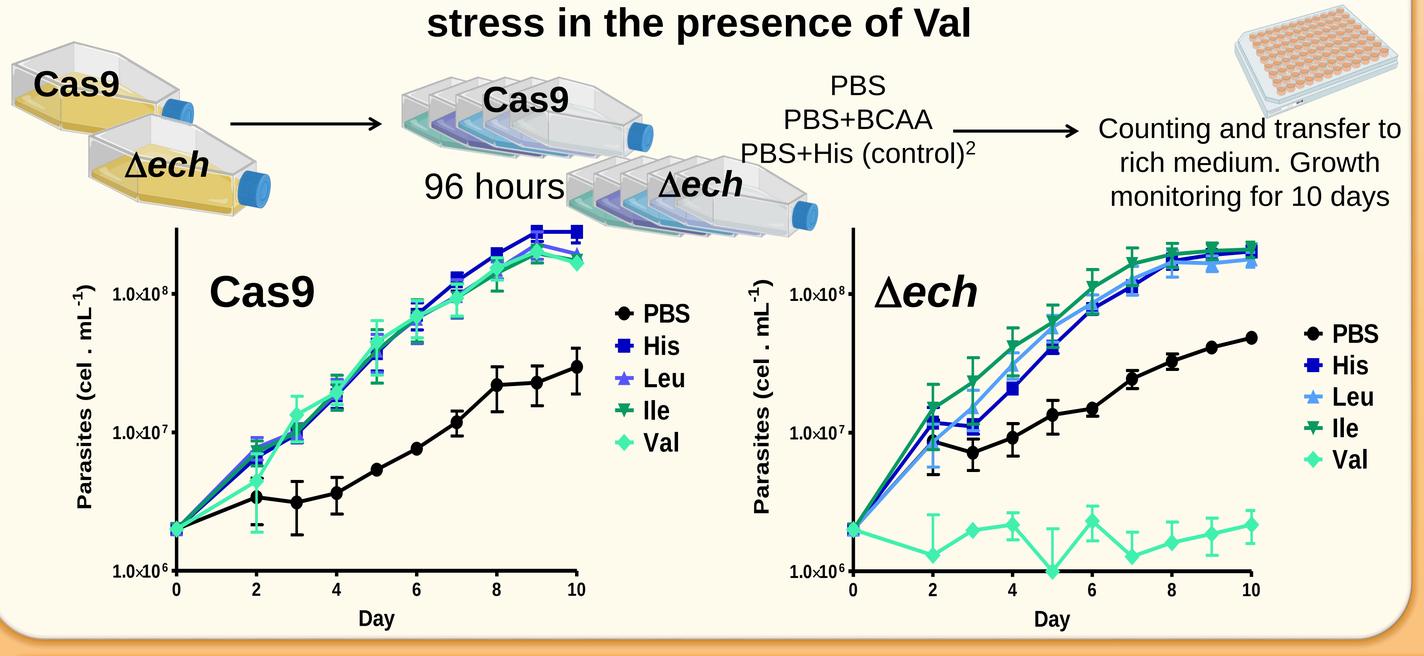


3. Δ*tcech* parasites have a diminished crotonase activity and are unable to degrade the Leu-related crotonoyl derivative

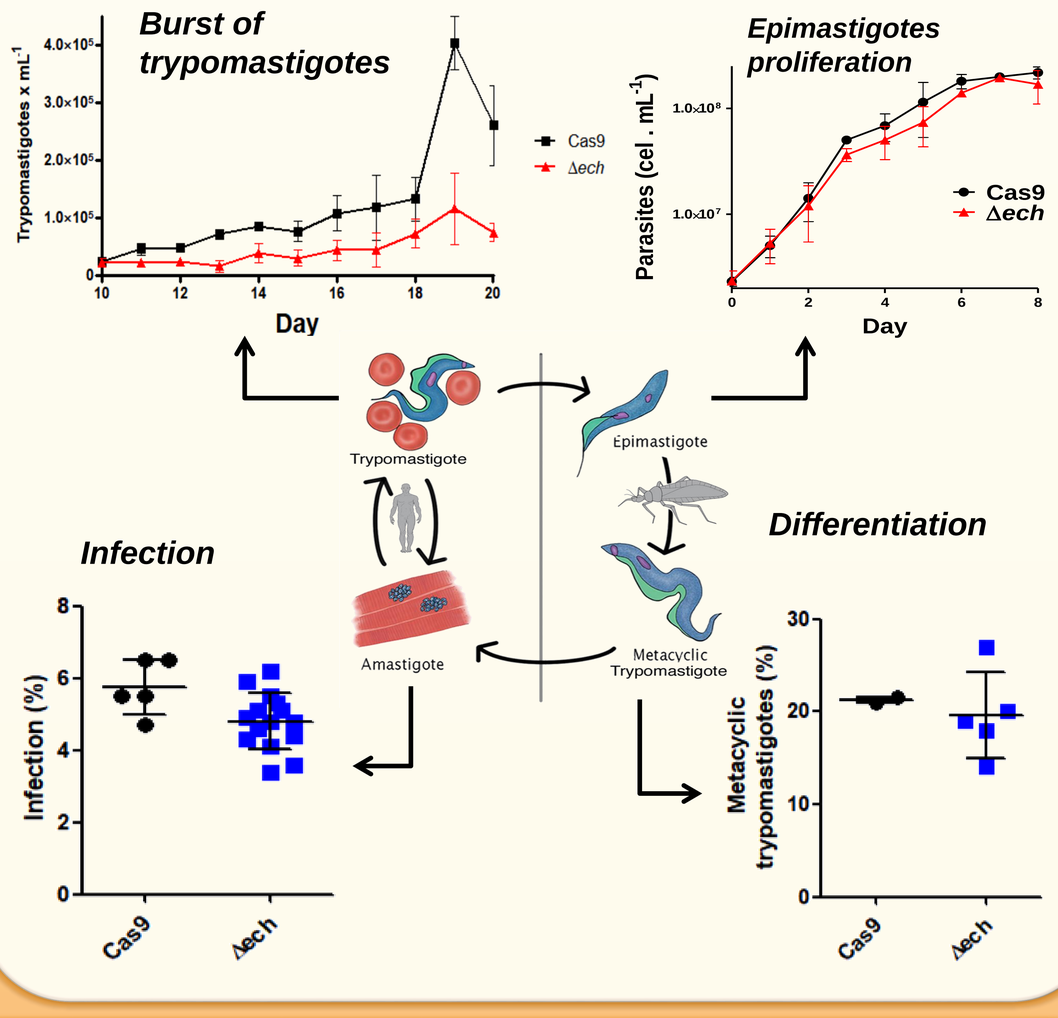
ECH enzymatic activity in Δ*ech* epimastigotes was tested using crotonoyl-CoA or methylcrotonoyl-CoA (specific Leu crotonoyl derivate) as substrates. When compared to the Cas9 expressing control cell line



4. Δ*ech* epimastigotes are not able to recover and die after nutritional stress in the presence of Val



5. Looking at the life cycle: Δ*tcech* parasites have a reduced trypomastigote bursting



Conclusions and future perspectives

In this work we cloned, expressed and initially characterized the recombinant enzyme TcECH. We obtained TcECH knockout epimastigotes by CRISPR-Cas9. The phenotypic analysis showed that Δ*ech* parasites have normal growth, can differentiate into metacyclic trypomastigotes and infect mammalian cells similarly to the control cell line. However, the absence of crotonase activity induce the accumulation of a toxic intermediate in the presence of Val and affect the intracellular cycle of the parasite. Further experiments will allow us to better understand the role of the BCAA oxidation pathway for the biology of *T. cruzi*.

Supported by

FAPESP FUNDAÇÃO DE AMPARO À PESQUISA DO ESTADO DE SÃO PAULO
CNPq
GCRF Global Challenges Research Fund

References

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2. Barisón MJ, Damasceno FS, Mantilla BS, Silber AM. 2016. The active transport of histidine and its role in ATP production in *Trypanosoma cruzi*. J Bioenerg Biomembr, 48(4).