Exometabolomic Analysis of Branched-Chain Amino Acid Catabolism Disruption in *Trypanosoma cruzi*

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Chagas disease remains a major public health concern in the Americas and, more recently, worldwide. *T. cruzi* exhibits remarkable metabolic flexibility, utilising amino acids not only as an alternative to glucose for carbon and energy but also for essential biological processes such as life cycle progression, stress adaptation, and host cell invasion. In this context, we are investigating the metabolism of branched-chain amino acids (BCAAs: leucine, isoleucine, and valine) in *T. cruzi* by analysing partial or complete knockout parasites for selected enzymes. These mutants were generated using CRISPR/Cas9 technology, targeting genes whose coding sequences are associated with this pathway in genomic databases.

Our goal is to gain deeper insights into the biological significance of the BCAA metabolic route in *T. cruzi*. Specifically, this study aims to elucidate how various metabolic pathways in the parasite interact with the BCAA catabolic pathway and how these interactions influence key cellular processes. To address our objectives, we analysed the exometabolome of *T. cruzi* knockout mutant lineages individually exposed to different carbon sources, aiming to elucidate the metabolic adaptations associated with BCAA utilisation. The secretion of metabolic by-products was evaluated by comparing knockout strains for specific enzymes with both the wild-type and parental (Cas9) strains.

Using ^{1}H-NMR-based exometabolomic analysis, we characterised the metabolic profiles of knockout mutants deficient in enzymes of the BCAA degradation pathway, including components of the branched-chain α-keto acid dehydrogenase complex (E1, E2, and E3), isovaleryl-CoA dehydrogenase (IVDH), and enoyl-CoA hydratase (ECH). Notable observations included elevated secretion of propylene glycol and formate, decreased acetone release, and modified levels of isobutyrate excretion. Our results reveal a metabolic interdependence among amino acid pathways in *T. cruzi*, emphasising the crucial role of enzymes involved in BCAA degradation. Most metabolic alterations were consistent with the targeted pathway disruptions; however, some metabolites, such as formate and propylene glycol, showed unexpected variations. These shifts suggest the occurrence of metabolic cross-talk with other pathways or compensatory mechanisms that enable adaptive responses to perturbations. Altogether, these insights highlight the complex metabolic network operating in *T. cruzi* and may guide the discovery of novel therapeutic targets against the parasite.