Identification of protein content released by the G and Y strains of the extracellular amastigote form of *Trypanosoma cruzi*

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INTRODUCTION

Trypanosoma cruzi possesses a complex life cycle, with different infective and invasive forms in different hosts. Among these infective forms, extracellular amastigotes (EAs), present in mammalian hosts, have been shown to be able to invade host cells without the need to differentiate back into trypomastigotes. Previous studies showed that these same parasite forms release vesicles and other molecules, in the presence of the host cell, which may be associated with the process of invasion and modulation of infectivity. These events were observed in previous results, in which vesicles and other released molecules from the G strain (highly infective) positively modulated the Y strain (low infective) host cell invasion in vitro and vice-versa.

AIMS

This study aims to identify molecules released by extracellular vesicles and investigate their importance for the invasion of different strains of *T. cruzi* with different levels of infectivity in host cells.

MATERIALS AND METHODS

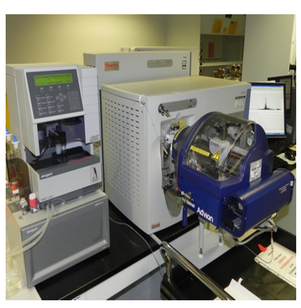
Extracellular vesicles

Ultracentrifugation

V2 | V16 | Vesicle Free (VF)

Particles size measurement NanoSight NS300

Liquid Chromatography /Mass Spectrometry (LC/MS-MS) analysis



Peptide and protein Identification

Gene ontology analysis

AmiGO 2

UniProt

NCBI Conserved Domains

Pfam

RESULTS

Size Measurement of Extracellular Vesicles

Fraction	Strain	Vesicles average size (nm)
V2	G	183
V16	G	92
V2	Y	188
V16	Y	88

Table 1. The vesicles in different fractions assessed relative to their sizes, showed that V2 fractions (obtained after 2 hours of centrifugation) had an average size twice as big in comparison to V16 (obtained after 16 hours of centrifugation) in both strains.

Virulence Proteins Content Released from *T. cruzi* G and Y strains

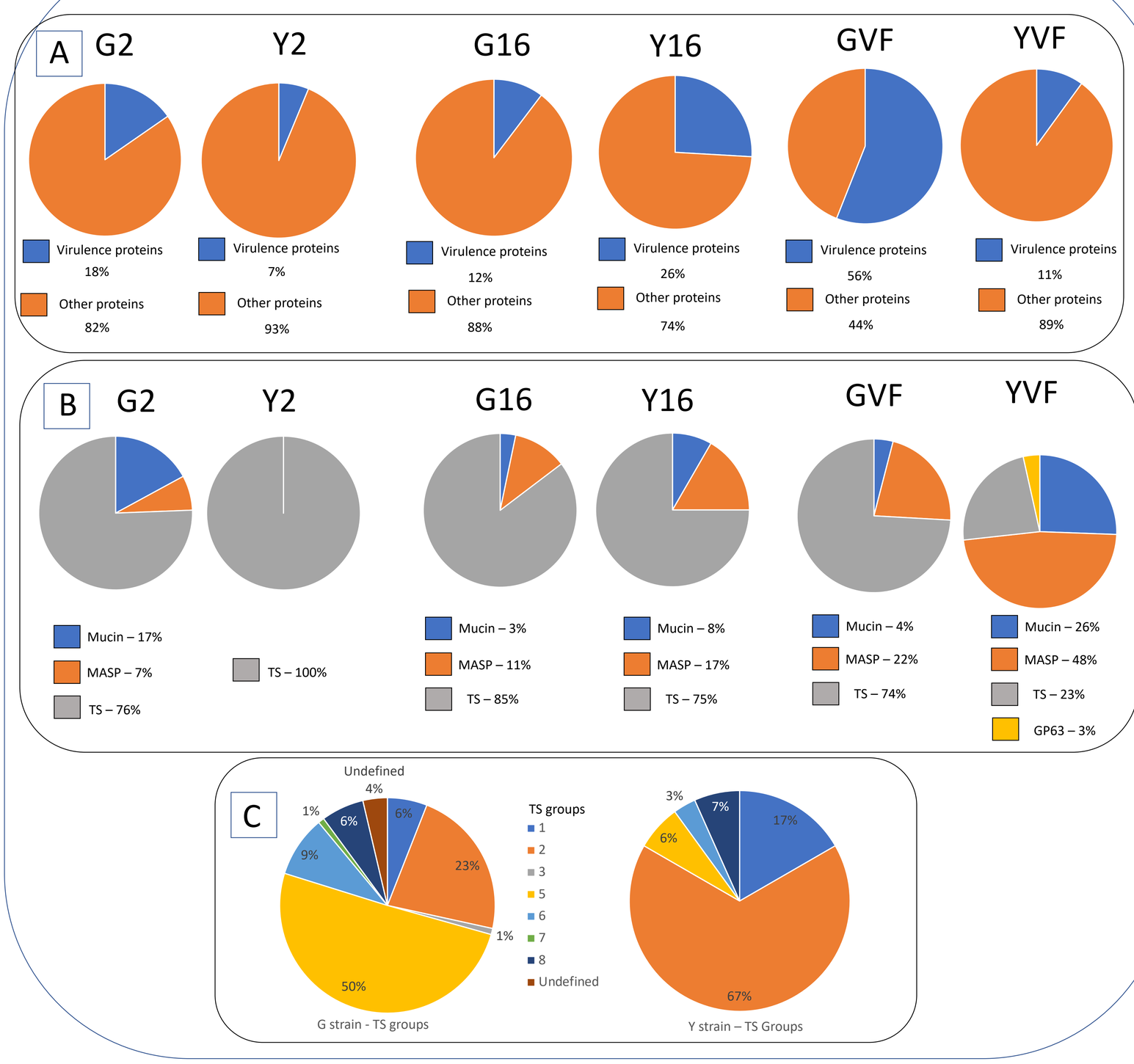


Fig 1. Virulence Proteins Content Released from *T. cruzi* G and Y strains. (A) When observing the whole protein content in each fraction, virulence proteins tended to be more present in G strain than in Y strain, specially in the vesicle free fraction. (B) Focusing on the virulence proteins in each fraction, mucins, mucin-associated surface protein (MASPs) and trans-sialidases (TS) were identified, with the TS being top hit in each fraction, excluding the Y strain VF fraction with MASP being the top hit and GP63 was also identified. (C) Despite TS being the virulence protein top hit in both strains, when observing the TS category groups, they showed quite different content, with TS-group 5 representing 50% of the TS found on G strain released content, while in Y strain this group was only 6% of the total TS amount. TS-group 2 was the most present in Y strain released content with 67% of the total, when compared to 23% of the G strain.

CONCLUSION AND PERSPECTIVES

The fact that more virulence factors are released by the G strain corroborates with previous studies that reported this strain to be more virulent than the Y strain in the extracellular amastigote form. Notably, despite TS being the top hits in both strains, they showed quite different composition when observing TS specific subsets of proteins. Therefore, the next steps of this study, will be the knockout through the CRISPR/CAS9 method of representative proteins of these groups, followed by host cells invasion assays. The presence of other molecules inside the vesicles (e.g., microRNA) are also being performed to identify other components that might also be involved in modulating the host cell during the infection process. The results from these analyzes will contribute for the identification of the main components released by the parasite during the invasion process and will lead to a better understanding of the invasion mechanism of *T. cruzi* extracellular amastigotes.

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FAPESP