# Heterologous expression of a neurotoxin from *Tityus serrulatus* scorpion venom in *Pichia pastoris* yeast and the evaluation of its glycosylation patterns

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## INTRODUCTION

*Tityus serrulatus* is the most dangerous species of scorpion in Brazil. Its venom (TsV) has mainly neurotoxins, which can on sodium or potassium channels and are responsible for most envenoming symptoms. The evaluation of these toxins can elucidate their mechanisms as well as contribute to a more specific therapy.

## OBJECTIVES

The aim of this study was the expression of Ts15, an α-KTx from TsV, in *Pichia pastoris* yeast, and its preliminary characterization.

## METHODS

rTs15 gene was synthetized by GenScript® with TEV (tobacco etch virus) protease cleavage site before N-terminal sequence and cloned into pPICZαA vector. The recombinant plasmid was transformed in KM71H *Pichia* strain and the screening of positive colonies was performed in deep well plate. The laboratorial scale expression was first performed with glycerol medium and methanol medium for the induction. The peptide expression was analysed by SDS-PAGE (16%) with silver stained and Schiff reagent that stain specifically carbohydrates. We also did a spectrometry analysis of toxins in MALDI-TOF equipment, a N-glycosylation reaction with PNGase enzyme and electrophysiological analysis in Kv 1.1, 1.2, 1.3 and 2.1 using the two-microelectrode voltage clamp technique.

## RESULTS AND CONCLUSIONS

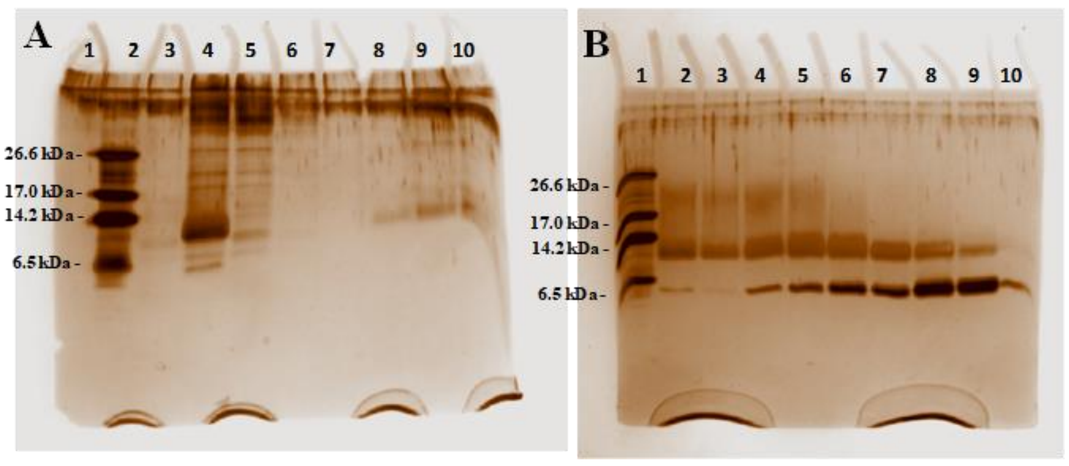


**Figure 1. (A and B). SDS-PAGE of fractions from IMAC (10 to 250 mM of imidazole).**

## RESULTS AND CONCLUSIONS

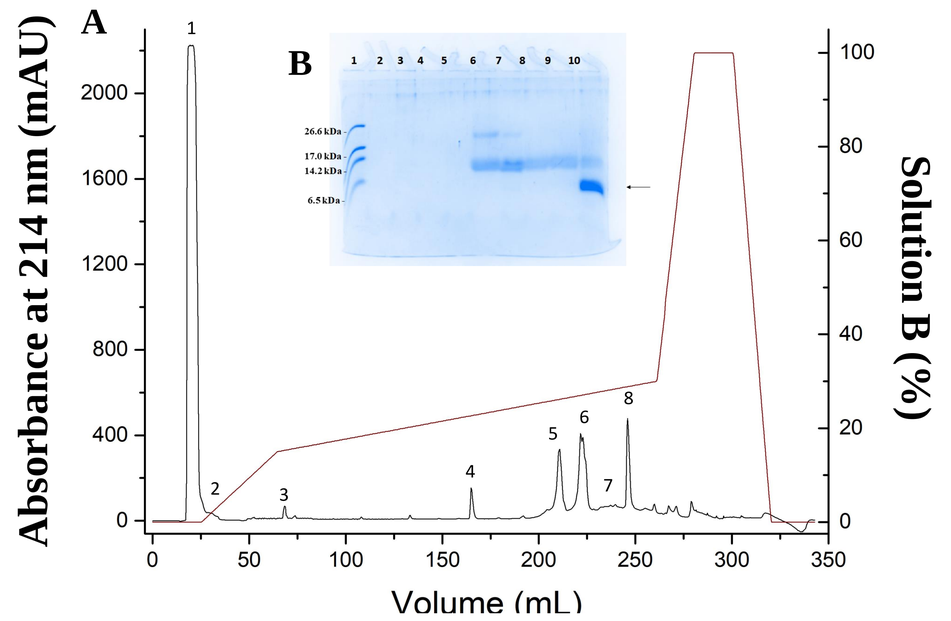


**Figure 2. (A) Reversed phase chromatography of IMAC fractions. (B) SDS-PAGE of fractions from reversed phase. 1:**Molecular weight marker; **3-10:** fractions 1 to 8. The arrow indicates that fraction 8 has the expected molecular mass for Ts15.

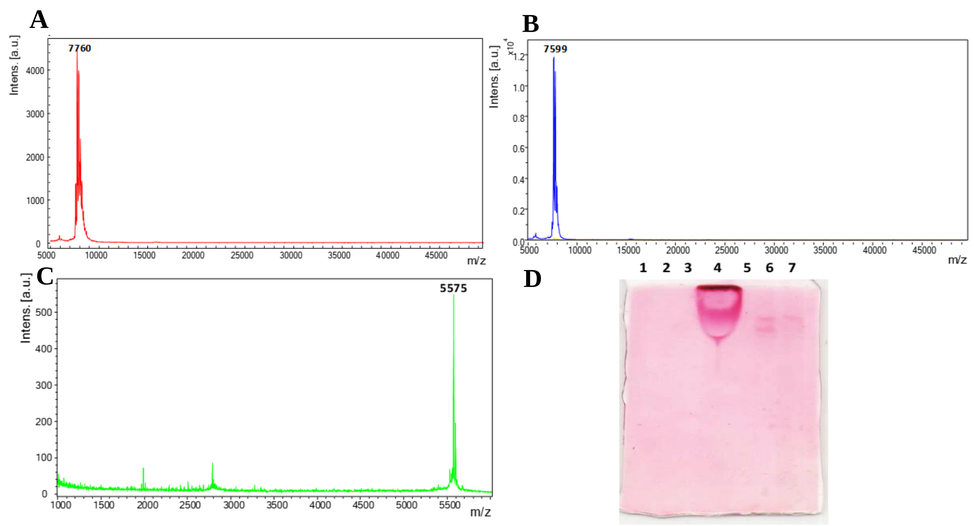


**Figure 3. (A to C). Mass spectra of 5, 6 and 8 fractions (respectively) from reversed phase in MALDI-TOF positive linear mode. (D) SDS-PAGE stained with Shiff reagent for glycoproteins. 1 and 2:** empty; **3:** Ultra-low molecular mass marker; **4**: albumin, **5**: rTs15 (fraction 8); **6:** fraction 5**, 7:** fraction 6.

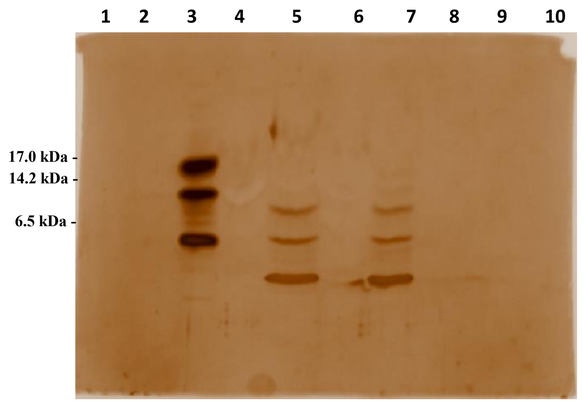


**Figure 4. SDS-PAGE after PNGase reaction. Lanes 1 and 2: empty; Lane 3: Ultra-low marker; Lane 4: fraction 5; Lane 5: fraction 5 deglycosylated; Lane 6: fraction 6; Lane 7: fraction 6 deglycosylated; Lanes 8 to 10: empty**

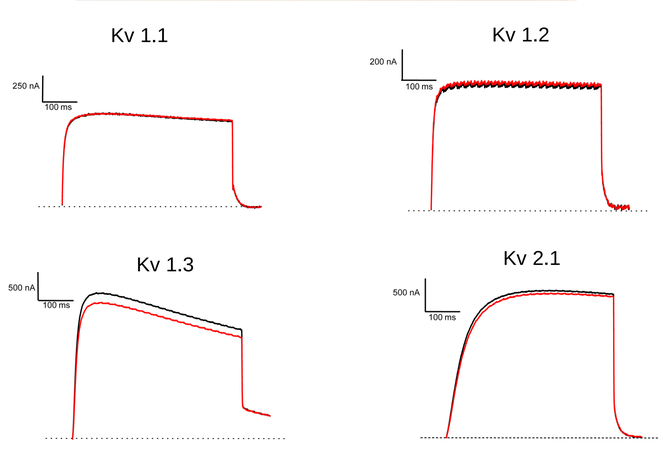


**Figure 5. Electrophysiology of fraction 8 (1 µM) on $K_V$s 1.1, 1.2, 1.3 and 2.1 channels.** The preliminary electrophysiological screening showed a small inhibition (6.8% ) on Kv1.3 current..

In conclusion, the rTs15 was successfully expressed in *P. pastoris* yeast, as well as two glycosylated forms of toxin, and the small inhibition in Kv 1.3 is probably due to the recombinant N-terminal. As next steps, the same tests will be performed with glycosylated and after cleaved toxins.

## FINANCIAL SUPPORT

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