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Expression and purification of rTs7, a recombinant toxin from *Tityus serrulatus* scorpion venom

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INTRODUCTION

Tityus serrulatus venom is composed of several substances, including the neurotoxins that interact with ion channels. These channels are involved in many diseases, such as arrhythmia, autoimmune diseases, hypertension and immune response to infections making *T. serrulatus* venom a source of biological tools to study them. The Ts7, acts selectively on potassium channels, and can contribute to the treatment of Kv1.3 channel-related diseases, such as autoimmune diseases.

OBJECTIVE

In this work, we present the heterologous expression of Ts7 in *Pichia pastoris* yeast and its purification.

METHODS

The gene was synthetized by GenScript® with TEV (tobacco etch virus) protease cleavage site before N-terminal sequence and cloned into pPICZαA vector. The *P. pastoris* cells (KM71H strain) were transformed with the linearized plasmid rTs7_pPICZαA. Transformation was confirmed by PCR. Positively transformed colonies were submitted to a screening in a 24-wells plate, under standard conditions (pH 6, for 144 h). And after, the best of them was subjected to laboratorial-scale expression, monitored by SDS-PAGE. The expressed protein was purified by reversed-phase chromatography, on a C-18 column. Three fractions were observed and, analyzed using mass spectrometry.

CONCLUSIONS

The rTs7 was successfully expressed and purified, with a high yield of the recombinant toxin, which showed similarity with the native toxin, and its immunosuppressive activity in multiple sclerosis model will be further investigated.

SUPPORT

CNPq FAPESP CAPES

RESULTS

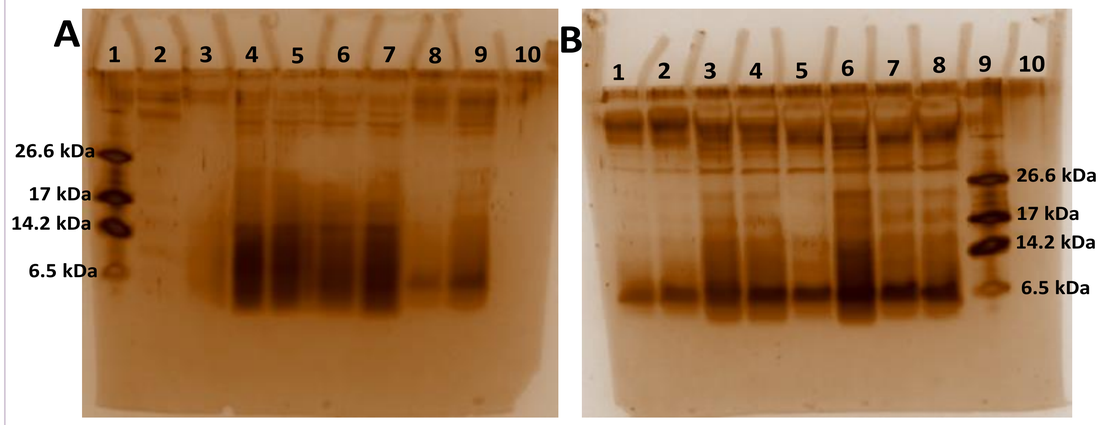

Figure 1: SDS gel with the screening of positive colonies. (A) 1: Molecular weight marker; **2:** negative control; **3:** positive control, **4-9:** times 0 h, 24h and 48 h of the colonies 7 and 21 **(B) 1-8:** times 72-144 h of the colonies 7 and 21; **9:** Molecular weight marker.

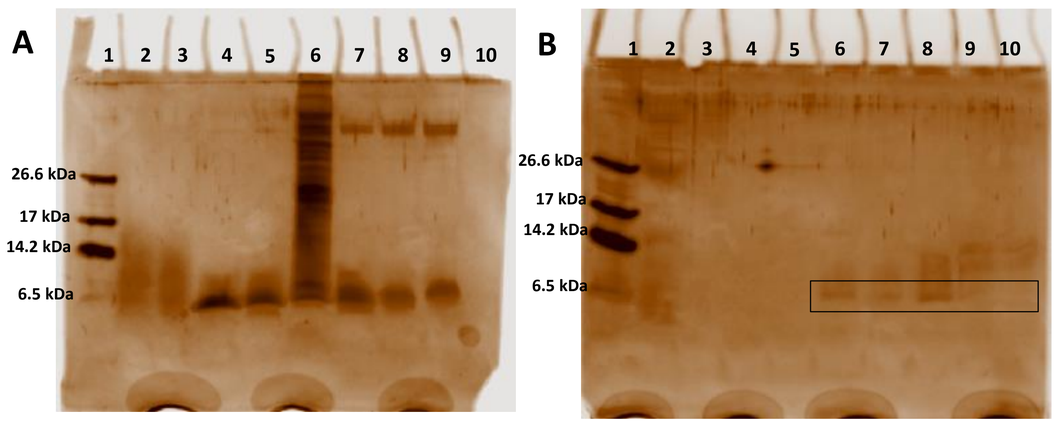

Figure 2: Laboratorial-scale expression and purification of rTs7. (A) The expression fractions at 0-144 h. 1: ultra low molecular mass pattern; **2:** negative control; **3-9:** times 0h-144h. **(B) Fractions from IMAC. 1:** ultra low molecular mass pattern; **2:** VOID; **3:** Washed; **4-10:** fractions from IMAC (10 mM to 250 mM of imidazole).

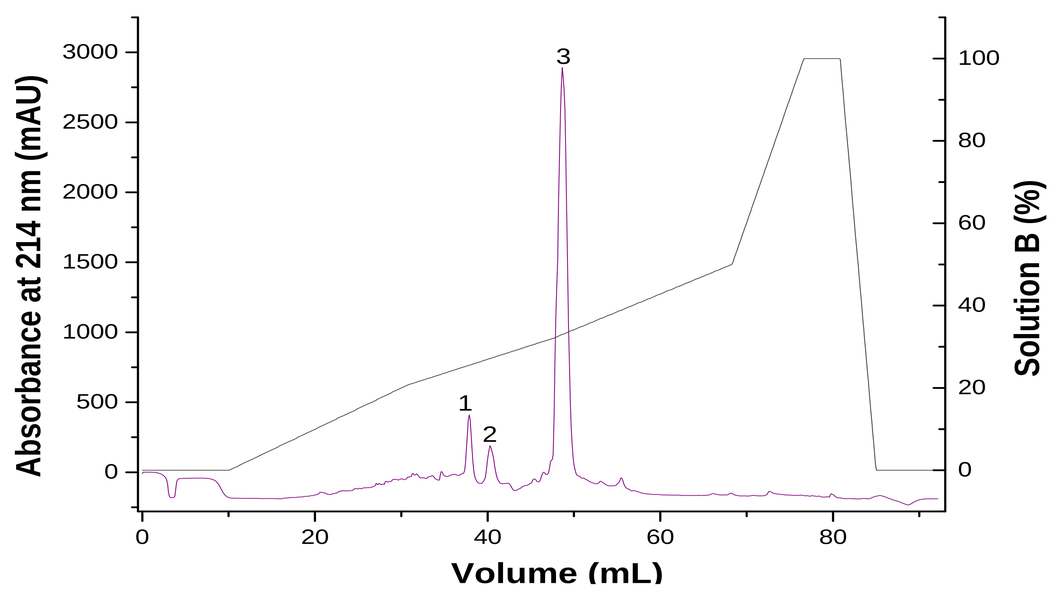

Figure 3: Reversed phase chromatography of IMAC fractions. Monitored by the FPLC Äkta UPC 900 system at 214 nm. The fraction was applied to the analytical C18 column (250 x 4.6 mm, with 5 µm particles, Jupiter, Phenomenex) previously equilibrated with 0.1% TFA.

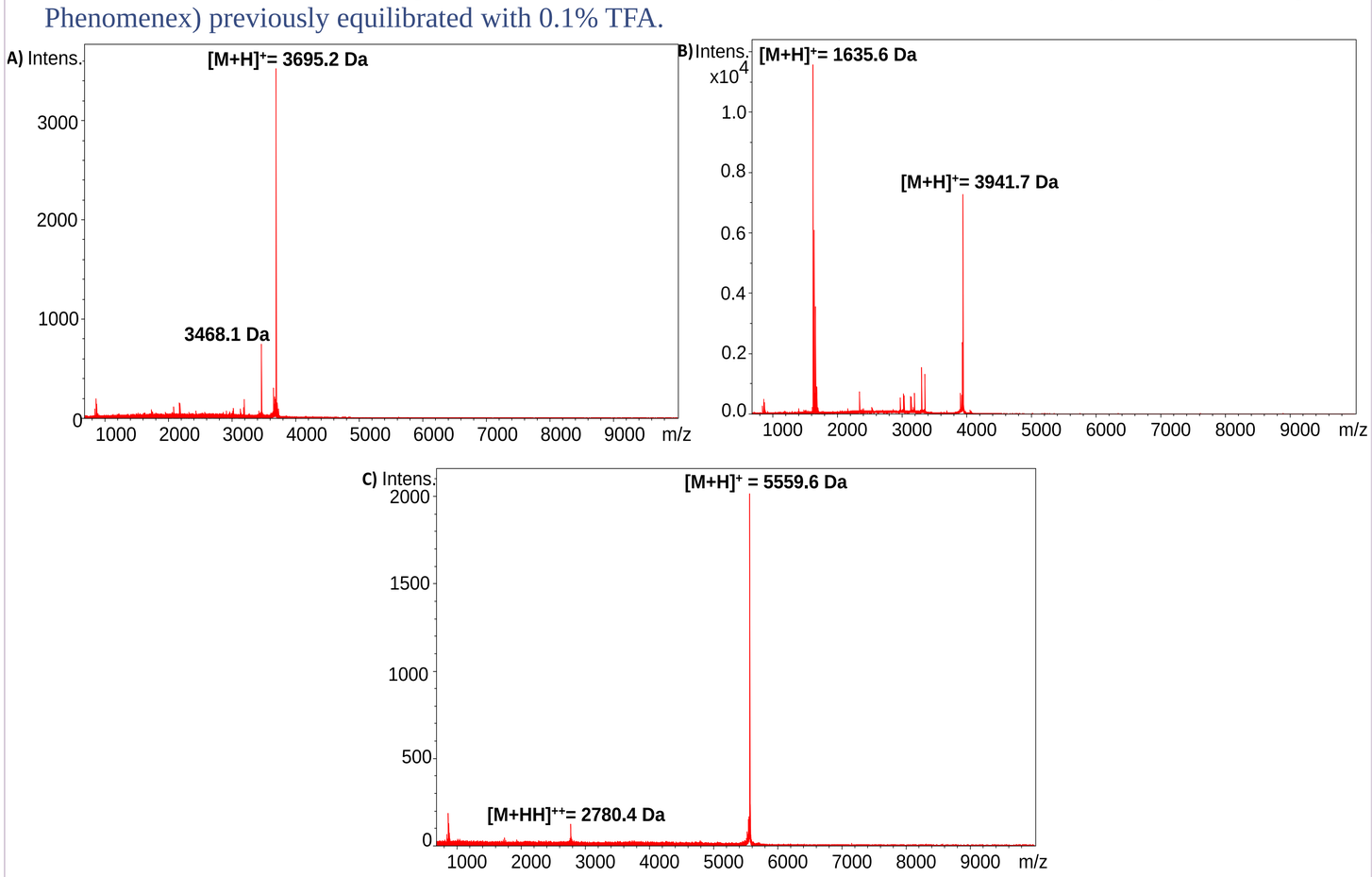

Figure 4: (A to C). Mass spectra of 1, 2 and 3 fractions from reversed phase in MALDI-TOF positive linear mode.