Title: ANALYSIS OF ACTIVITY PEPTIDE FROM SEEDS *Capsicum chinense* WITH SEQUENCE HOMOLOGOUS TO TRYPSIN INHIBITOR AGAINST *Staphylococcus aureus* AND *Escherichia coli*.

Authors: Zottich, U.[1]; Gomes, V.M. [2]; Dias, G.B.[3]; Vallorini, T.P.C.[1]; Schuenck, R.P.[1]*

Institution: 1* Laboratório de Biologia Molecular e Virulência Bacteriana - Departamento de Patologia - Universidade Federal do Espírito Santo – ES - Brazil.

2 Laboratório de Fisiologia e Bioquímica de Microrganismos/CBB – Universidade Estadual do Norte Fluminense – Campos, RJ - Brazil

3 Laboratório de Ecologia Vegetal – Universidade Vila Velha – ES - Brazil

*E-mail address: ricardoschuenck@yahoo.com.br

Abstract:

Different peptides have been isolated from a wide range of animal and plant species. Due to the development of multi-resistant microorganism, antibacterial and antifungal peptides have attracted the attention in recent years in order to find new therapeutic agents. Various peptides, which include trypsin inhibitor, play an important role in the protection of plants against microbial infection. In this study, peptides from *Capsicum chinense* seeds were extracted and recovered by freeze drying, diluted in 0.1% (v/v) trifluoracetic acid (TFA) and injected onto an HPLC Vydac C18 reverse phase column (250 × 4.6 mm). The solvent flow rate was 0.5 mL/min and the solvent progressed from 100% solvent A (0.1% TFA in water) for 10 min, 0 to 50% solvent B (100% 2-propanol containing 0.1% TFA) for 50 min, 50% solvent B for five min and finally returned to 0% of solvent B for 10 min. Elution of proteins was monitored by on-line measurement of the absorbance at 220 and 280 nm. The peptide enriched fraction, namely RP1, was obtained. The RP1 fraction composed of one peptide ranging from 6 kDa with homology to trypsin inhibitor. To monitor the effect of RP1 fraction on the bacterial growth, 10^6 UFC/mL cells were incubated in 200 µL of Mueller Hinton cation adjusted broth in presence of the peptide (0, 12.5, 25 and 50 µg/mL) at 35 °C. Optical readings at 620 nm were taken at the initial time point and every 2 h until 24 h. This fraction exhibited antibacterial activity of 67% in concentration at 50 µg/mL of RP1, demonstrating inhibition against *Escherichia coli* Dh5α after 12 hours of growth. On the other hand, in *Staphylococcus aureus* ATCC 29213 it had stimulated their growth. However, higher concentrations of peptide are necessary to provide new information about the mechanism of action. This way, we suppose that RP1 fraction is a promising antibacterial and acts in the signaling process and toxicity of the peptide-bacterial interaction, whose mechanism of action may involve steps of membrane permeabilization, characteristic this class of peptide.

Keywords: Trypsin inhibitor, *Capsicum chinense*, antimicrobial peptides.

Financial support: FAPES/CAPES/CNPq