ANALYSIS OF EXPRESSED SEQUENCE TAGS (EST) OF AVOCADO SEED (*Persea americana* var. drymifolia) REVEALS ABUNDANT EXPRESSION OF THE ANTIMICROBIAL PEPTIDE SNAKIN GENE

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Introduction. Avocado is one of the most important fruits in the world. Avocado "native mexicano" (*Persea americana* var. drymifolia) seeds are very important for the propagation of this plant (1). This is mainly because their seedlings have resistance to *Phytophthora cinnamomi* attack and are adapted to the soil and environmental conditions of the region. Recent evidence indicates that both plants and animals share some features in their defense strategies, including the production of antimicrobial peptides (AMP).
In this work we reported a set of ESTs from avocado "native mexicano" obtained from seeds and identified a clone encoding the AMP snakin gene. The AMP members of the snakin/GASA family also have effects on innate immunity (2,3).
The aim of this work was to express this gene in a bovine endothelial cell system and evaluate its antimicrobial activity against animal and human pathogens.

Methods. cDNA libraries were built from 1 µg of total RNA using SMART™ cDNA library construction kit (Clontech). The obtained cDNA fragments were cloned into TripIEx2 vector. Excision experiments were made using *E. coli* BM25.8 cells to obtain the plasmid pTriplEx2. Sequencing reactions were performed using ABI PRISM BigDye Terminators v3.0 kit (Applied Biosystems). PaSn clone was identified and *PaSn* cDNA was cloned into the mammalian expression vector pTracer-EF/V5-His-A (Invitrogen) to obtain the pPaSn construction (Fig. 1A), which was expressed in the bovine endothelial cell line BVE-E6E7. Conditioned media (CM) from polyclonal and clonal populations were used to evaluate their activity against *Escherichia coli* and *Staphylococcus aureus* by MTT assay.

Results. In this work we reported the isolation of 5,005 ESTs from 5' ends of the cDNA clones representing 1,653 possible unigenes, which were obtained from *P. americana* var. drymifolia seed. Avocado AMP snakin was one of the most abundant seed messenger. We expressed the snakin gene in BVE-E6E7 cells and CM from transfected cells showed antimicrobial activity against *E. coli* and *S. aureus* strains, with inhibition percentages higher than 90% in both cases (100 µg/ml total protein, Fig. 1B).

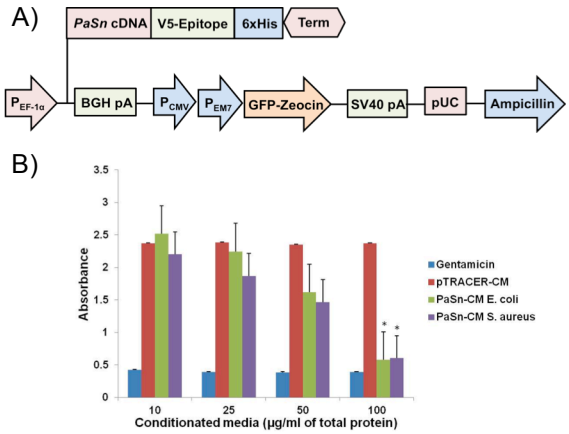

Fig. 1. A) pPaSn construction. B) Antimicrobial activity of CM from BVE-PaSn cells on *E. coli* and *S. aureus*. (*) Show significant differences (P< 0.05).

Conclusions. We report the analysis of the ESTs obtained from a cDNA library of avocado seed and identified the AMP snakin. This AMP showed antimicrobial activity against *E. coli* and *S. aureus*. Our data suggest that avocado snakin PaSn could be involved in the seed defense as part of the plant innate immune system.

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