ANTIBACTERIAL ACTIVITY OF DEFENSIN PaDef FROM AVOCADO FRUIT (*Persea americana* var. drymifolia) EXPRESSED IN ENDOTHELIAL CELLS AGAINST *Escherichia coli* AND *Staphylococcus aureus*

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Introduction. Antimicrobial therapy is a useful tool to control diseases, clinical activity that has originated an increase in resistant microorganisms. Alternative strategies are desirable and antimicrobial peptides (AMP) represent attractive control agents (1). Mexican avocado (*Persea americana* var. drymifolia) is used in traditional medicine; however, the AMP production has not been reported in this plant.
Therefore the aim of this work was to express the AMP PaDef from avocado in bovine endothelial cells and evaluate its antimicrobial activity against different pathogens.

Methods. We obtained a cDNA library from avocado mesocarp using SMART™ cDNA library construction kit (Clontech) and clone PaDef was identified. *PaDef* cDNA was cloned into the mammalian expression vector pTracer-EF/V5-His-A (Invitrogen), and the construction (pBME3) was used to transfect the bovine endothelial cell line BVE-E6E7 by lipofection. Polyclonal and clonal populations were selected and the conditioned media (CM) were used to evaluate their activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* by MTT assay.

Results. Sequence analysis of PaDef clone revealed that has a cDNA of 249 bp encoding a protein of 78 aa homologous with AMP plant defensins (>80%). This putative protein contains a signal peptide of 31 aa, which when is removed produces a mature peptide of 47 aa. A comparative study with plant defensins showed that mature peptide contains the conserved 8 cysteines, which could form the 4-disulfide bridges characteristic of this AMPs (Fig. 1A). pBME3 construction was used to express defensin *PaDef* cDNA in BVE-E6E7 (Fig. 1B). We tested the activity of CM from polyclonal (BVE3PC) and clonal populations (BVE3C1-9) against *E. coli*, *S. aureus* and *C. albicans* (Table 1). *E. coli* viability was inhibited with 100 µg/ml of total protein from clones (>80%). Also, *S. aureus* viability was inhibited from 50 µg/ml total protein (30-39%), but was more evident at 100 µg/ml (62-67%). Finally, we did not detect activity against *C. albicans.*

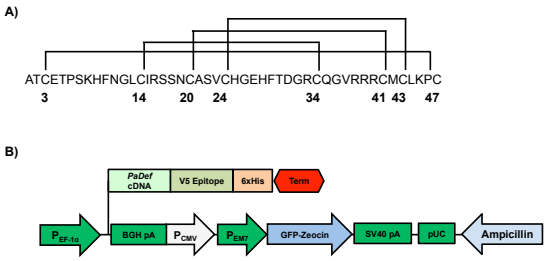

Fig. 1. A) Prediction of disulfide bridges of defensin PaDef. B) The pBME3 construction.

Table 1. Antibacterial effect of CM from BVE-E6E7 cells that express defensin PaDef.

	Inhibition (%)							
	E. coli				*S. aureus*			
	CM (total protein, µg)				CM (total protein, µg)			
Clones	10	25	50	100	10	25	50	100
BVE3PC	6	14	1	20	11	10	25	24
BVE3C1	9	1	11	74*	5	4	39*	64*
BVE3C2	10	10	11	70*	17	13	34*	64*
BVE3C3	13	1	7	76*	12	14	31*	67*
BVE3C4	24	15	15	65*	10	15	33*	55*
BVE3C5	22	16	12	76*	14	6	30*	65*
BVE3C6	20	16	8	78*	13	11	31*	64*
BVE3C7	14	13	21	80*	12	4	31*	60*
BVE3C8	22	13	10	71*	16	11	33*	58*
BVE3C9	16	15	9	56*	1	14	27*	52*

* Indicates significant changes ($P < 0.05$) compared to CM from BVE-E6E7 cells.

Conclusion. This is the first report that shows antimicrobial activity of a defensin produced by avocado fruit, which suggest that could be use in the treatment of infectious diseases.

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