

## FICHA DE TESIS

<b>Nombre del graduado</b>	Eduardo Andrés Tapia Rodríguez
<b>Año de ingreso al programa</b>	2009
<b>Título de la tesis</b>	Desarrollo y evaluación de vectores de expresión inducibles para el uso biotecnológico de péptidos antimicrobianos.
<b>Director(a) de tesis</b>	Gloria Arenas Díaz
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<b>Publicaciones</b>	<b>Indexada (identificar tipo de indexación: ISI, SCIELO, LATINDEX, u otra):</b> <i>Autor(es), año, nombre, lugar, editorial, estado, ISSN. Incluir factor de impacto de revista, si es pertinente.</i> 1. <b>Tapia, E.</b> , Montes, C., Rebufel, P., Paradela, A., Prieto, H., Arenas, G. (2011) Expression of an optimized <i>Argopectenpurpuratus</i> antimicrobial peptide in <i>E. coli</i> and evaluation of the purified recombinant protein by in vitro challenges against important plant fungi. PEPTIDES 32:1909-1916
	<b>No indexada:</b> <i>Autor(es), año, nombre, lugar, editorial, estado</i>
<b>Patentes:</b>	1. Chimeric gene for heterologous expression that encodes peptides with antimicrobial activity (2013) <b>Eduardo Tapia</b> , Christian Montes, Humberto Prieto, Gloria Arenas. US13/784,243.
	2. Gen químérico para expresión heteróloga que codifica para péptidos con actividad antimicrobiana (2012) <b>Eduardo Tapia</b> , Christian Montes, Humberto Prieto, Gloria Arenas. 2012-00593.
<b>Resumen de la Tesis</b> <p>Antimicrobial peptides (AMP) have been widely described in several organisms from different kingdoms. We recently designed and evaluated a synthetic version of an AMP isolated and characterized from <i>Argopectenpurpuratus</i> hemocytes. This study describes the generation of a chimaeric gene encoding for Ap-S, the use of this construct to transform <i>E. coli</i> strain BL21, and the evaluation of the purified recombinant Ap-S (rApS) as an antifungal agent. The proposed gene coding for rAp-S consists of 93 nucleotides arranged downstream from the IPTG-inducible T7 promoter. The best synthesis conditions were obtained after <i>E. coli</i> cultivation at 26 °C for 3 h, which allowed for the production of an rAp-S-enriched fraction containing the peptide at 249 nM. Mass spectrometry analysis of the purified rAp-S (3085.80 Da) showed the addition of a glycine residue on its N-terminal end derived from vector design and peptide purification. The purified rApS fraction was assayed for antifungal activity by direct addition of purified rApS elution to potato dextrose agar media at a final concentration of 81 nM. These assays showed important growth inhibitions of both biotrophic (<i>Fusariumoxysporum</i>, <i>Trichodermaharzianum</i>) and necrotrophic (<i>Botrytis cinerea</i>, <i>Alternaria</i> spp.) fungi in that the hyphae structures and spore count were affected in all cases. The strategy of cloning and expressing rAp-S in <i>E. coli</i>, the high yield obtained and its successful use for controlling pathogenic fungi suggest that this</p>	

molecule could be applied to agricultural crops using various management strategies.