Transient expression of GFP-Pg-AMP1 by Agrobacterium tumefaciens in Nicotiana benthamiana.


1 Center for Proteomics and Biochemical Analysis, Pos-Graduation Program in Molecular Pathology, University of Brasília, Brasília-DF, Brazil; 2 EMBRAPA Genetic Resources and Biotechnology, Brasília-DF, Brazil; 3 Center for Proteomics and Biochemical Analysis, Pos-Graduation Program in Genomic Sciences and Biotechnology, Catholic University of Brasília, Brasília-DF, Brazil; e-mail presenting/corresponding author: juliane.viana@gmail.com.br

Antimicrobial peptides represent an alternative to antibiotics currently utilized. However, the low production costs of large quantities of purified and active biomolecules are extremely necessary for possible pharmaceutical use. An alternative system for the heterologous production of proteins and peptides consists in the transient expression in plants using Agrobacterium-mediated transformation. In this report, the synthetic gene was constructed using the primary sequence of the antimicrobial peptide Pg-AMP previously isolated and purified from guava (P. guajava) seeds containing an extra N-terminal histidine-tag (6xHis) fused to a chloroplast signal peptide in a pCambia2300 binary vector. The green fluorescent protein (GFP) was fused to the peptide N-terminal and the resultant vector was transferred to Agrobacterium tumefaciens EHA105. Nicotiana benthamiana leaves were further infiltrated using a syringe needleless and collected after 4 days. Plant crude extract was analyzed by SDS-PAGE 15% and Pg-AMP1 was recognized by Western-blot analyses using anti-GFP polyclonal antibody, showing a molecular mass of 33 kDa. The transient expression in N. benthamiana by agroinfiltration leads to an efficient Pg-AMP1 production. In summary, this system may be used for small scale production of the GFP-PG fusion allowing further purification and bioassays against the pathogenic bacteria Proteus sp. and Klebsiella sp. Those expressed peptides could be utilized in a near future to develop nanoparticles that could be used against bacteria cause hospital infections.

Keywords: Antimicrobial peptides, transient expression, Nicotiana benthamiana, hospital infections.

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