Biotecnología aplicada al mejoramiento genético, cultivo, conservación y propagación de plantas medicinales

POSTER 12

IDENTIFICATION OF A LIPID TRANSFER PROTEIN FROM ROMAN CHAMOMILE

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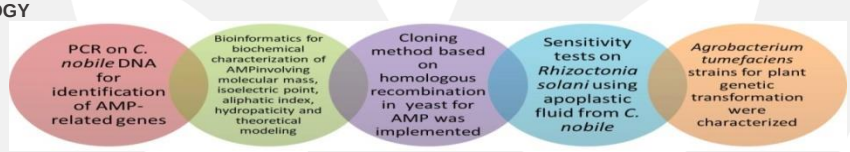
1. INTRODUCTION

Roman chamomile (*Chamaemelum nobile*) is a medicinal plant widely used because of flavonoids, sesquiterpenes and azulenes synthesis, which have anti-inflammatory, anticancer and antimicrobial properties. Phytopathogenic inhibitory properties of Roman chamomile against *Staphylococcus aureus*, *Enterococcus faecalis*, *Cándida albicans* o *Penicillium sp*. have been described (Dezfoil et al., 2012; Kazemian et al., 2015; Ghaedi et al., 2015). Antimicrobial peptides (AMPs) are a compound of plant innate immunity which increase their concentration in susceptible tissues as response against phytopathogenic invasion (Maróti et al., 2011). The aim of this work was to establish a model whose goal is the integration of molecular, bioinformatics and biological tests within the framework of AMP identification.

2. METHODOLOGY



3. RESULTS

Identification of an AMP gene called Lipid Transfer Protein (LTP) (94 amino acids) was made from genetic material of *C. nobile* using primers forward (5'-GACTGCTCAACGGTTCAGTAAAGTTGA-3') and reverse (5'-TCAACTTTACTGAACCGTTGAGCAGTC-3') (Figure 1A-B). The putative LTP sequence identified in *C. nobile* was deposited in GenBank (ID: MT294300.1) and characterized *in silico* (Figure 1C), so, *C. nobile* LTP is similar to Type1 family of these antimicrobial molecules containing 9.4 kDa, isoelectric point of 9.39, 56 aliphatic residues (aliphatic index of 87.34 representing a thermostable protein), 13 positives residues and three negative residues, an alpha helices structure, hydrophobic cavity . Homologous recombination in yeast was employed, and it was found that depends on the level of vector-genome homology (Figure 1D-E). Apoplast washing fluid (AWF) from *C. nobile* has activity against *R. solani* at 1 µg/mL (Figure 1F). Finally, a phenotypic (all of strains are rifampicin resistant, and LBA4404 is kanamycin resistant) and molecular characterization of *A. tumefaciens* strains that may be useful in the genetic transformation of plants was carried out as a perspective of plant transformation using *C. nobile* LTP-related gene for phytopathogen resistance (Figure 1G).

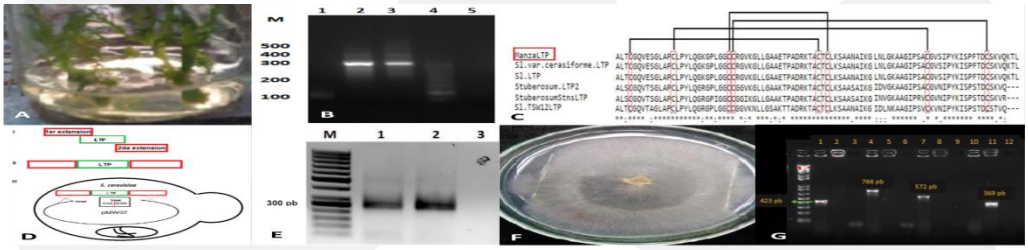


Figure 1. Identification of a LTP from *C. nobile*. A) *In vitro* culture (MS + Gamborg salts) of *C. nobile*; B) PCR of a LTP gene (300 bp); C) LTP sequence (red square) and other LTPs, bars indicate four conserved disulfide bonds; D) Homologous recombination in yeast scheme showing two rounds of amplification using a putative site from pMW07 vector; E) PCR from plasmid DNA from Saccharomyces cerevisiae; F) Bioassay on *R. solani* using *C. nobile* AWF; G) PCR of *A. tumefaciens* conserved genes, M: Marker of 1 Kb (Invitrogen TM). lane 1: Positive PCR of GlyA gene from C58-MP90 strain, lane 2: Negative PCR of GlyA gene using DNA from LBA4404 strain, lane 3: absolute control, lane 4: Positive PCR of pTiBo542 from EHA101 strain, lane 6: negative PCR of pTiBo542 gene using DNA from LBA4404 strain. lane 7: absolute control, lane 7: positive PCR of nptII gene using DNA from EHA101 strain, lane 8: negative PCR of nptII gene using DNA from LBA4404 strain, lane 9: absolute control, lane 10: negative PCR of Ach5FtsZ gene using DNA from EHA101 strain, lane 11: positive PCR of Ach5FtsZ gene from LBA4404 strain, lane 12: absolute control.

4. CONCLUSIONES

a) *C. nobile* LTP is an antimicrobial peptide of LTP1 family; b) C. nobile LTP has four disulfide bonds; c) Homologous recombination using yeast requires primers for RAD5 enzyme, d) *C. nobile* AWF has activity against R. solani at 1 µg/Ml, e) *A. tumefaciens* could be used for plant transformation using the LTP gene from *C. nobile*.

5. REFERENCES

- Dezfooili N, Hasanzadeh N, Rezaee M, Ghasemi A. Antibacterial Activity and Chemical Compositions of Chamaemelum nobile Essential Oil/ Extracts against Pseudomonas tolaasii, the Causative Agent of Mushroom Brown Blotch. Annals of Biological Research; 2012, 3 (6): 2602 – 2608.
- Ghaedi, M., Naghiha, R., Jannesar, R., dehghanian, N., Mirtamizdoust, B., & pezeshkpour, V. (2015). Antibacterial and antifungal activity of flower extracts of Urtica dioica, Chamaemelum nobile and Salvia officinalis: Effects of Zn[OH]2 nanoparticles and Hp-2-minh on their property. Journal of Industrial and Engineering Chemistry, 32, 353–359.
- Kazemian, H., Ghafourian, S., Heidari, H., Amiri, P., Yamchi, J. K., Shavalipour, A., … Sadeghifard, N. (2015). Antibacterial, anti-swarming and anti-biofilm formation activities of Chamaemelum nobile against Pseudomonas aeruginosa. Revista Da Sociedade Brasileira de Medicina Tropical, 48(4), 432–436.
- Maróti, G., Kereszt, A., Kondorosi, É., & Mergaert, P. (2011). Natural roles of antimicrobial peptides in microbes, plants and animals. Research in Microbiology, 162(4), 363–374