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Molecular Farming of Oral Vaccine: Phytosecretion of ETEC(987P, K88ac) Fimbrial Protein(FasG, Pili) from Transgenic Carrot Cells

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Objectives

We have developed an apoplast-localization vector for the most suitable expression and accumulation in plant cell. This may be used to enhance the feasibility of plants as commercial means for large-scale protein production.

Materials and Methods

1. Materials - plant: *Daucus carota* L. (Jochun5chon, Hanyeorum5chon, Mansan5chon)
   vector: pGA748, apoplast-localization vector (pGAspfasG, pGAsppili)
   medium: MS+(0.1*mg/l* 2iP, 1*mg/l* 2,4-D, 3% sucrose)
   MS-(MS medium, 0.1*mg/ml* 2iP, 2.5% sucrose)
2. Methods - Vector construction, *Agrobacterium*-mediated Transformation, PCR, Callus culture, SDS-PAGE, Western analysis, Secretion test

Results and discussions

1. It was shown that a fragment of gene for signal peptide(sp) from CLP(Chtinase Like Protein) was shown to be a minimum requirement for extracellular localization when fused to 5' end of target genes such as FasG and Pilin genes.
2. Introductions of the spFasG and spPilin gene were verified by PCR with spFasG or spPili primers showing the expected bands of 1042bp and 1396bp, respectively in transgenic carrot callus.
3. The recombinant spFasG and, spPili proteins were successfully targeted to be secreted in transgenic carrot callus, as demonstrated by the release of antigenically detectable levels in exudates surrounding callus cultured onto nitrocellulose membrane.
4. Secretion of the proteins into the liquid medium from callus suspension cells of spfasG, sppili transformant were also revealed by western analysis.

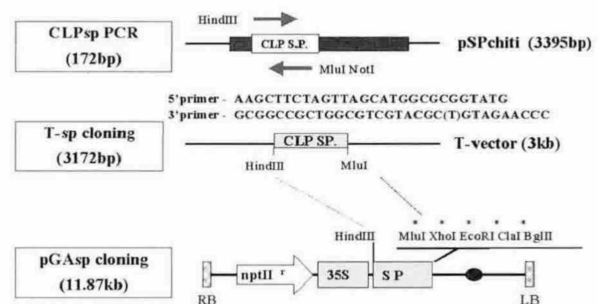

Figure 1. Schematic diagram showing the construction of pGAsp vector.

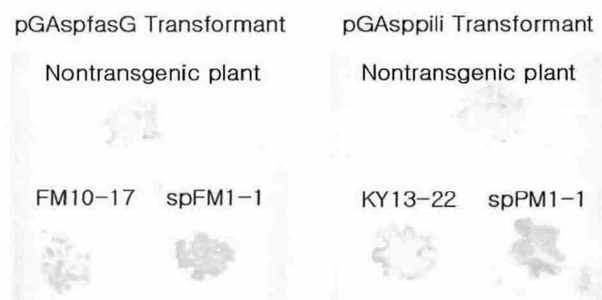

Figure 2. Transgenic and nontransgenic calli were placed onto a sterile nitrocellulose filter on a MS-agar media for 5days at 26°C and the filters were reacted with anti- FasG(987P) or Pilin(K88) antiserum.

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