

P 81

Immunogenicity of Enterotoxigenic *E. coli* (ETEC) 987P Plant Vaccine

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Objectives

The previous research showed that a pilin of ETEC K88ac expressed in carrot had been successful for oral immunization of piglet. We are going to report a cloning of fasG from enterotoxigenic *E. coli*, 987P and a development of the second transgenic carrots for oral vaccine against enterotoxigenic *E. coli*, 987P.

Materials and Methods

1. Materials

- plant: *Daucus carota* L. (Jochun5chon, Hanyeoreum 5chon, Mansan5chon)
- Vector: pGAfasG
- Medium: MS⁺(callus induction medium: 2.4-D 1 mg/L), MS⁻

(regeneration medium)

2. Methods: Vector construction, Tissue culture, Transformation (*Agrobacterium*-mediate), PCR, western

Results and discussion

1. Approximately 400 transgenic cell lines were selected in the MS medium containing a kanamycin
2. An introduction of the FasG gene was verified by PCR with FasG primer showing the expected bands of 1200 bp in transgenic carrot callus
3. We had selected the cell-line with a high level of expression by western analysis and regenerated plantlets.
4. An efficiency of oral immunization in mouse will be tested and a further possibility of transplacental antibody transfer to offspring of immunized pregnant mice will also be tested.

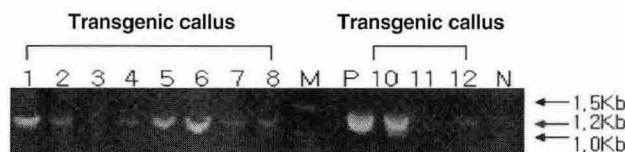


Figure 1. Confirmation of the fasG gene introduced in transgenic carrot cell by PCR using genomic DNA M, 100 bp ladder marker; P, fasg gene (1.2 Kb) of 987P cloned in pGA vector as a positive control; N, nontransgenic callus.

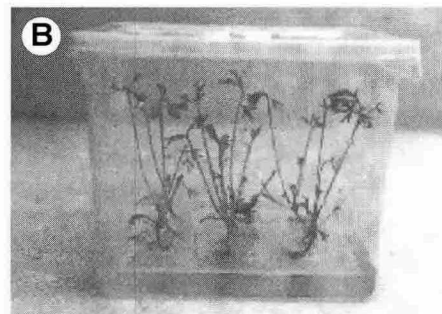
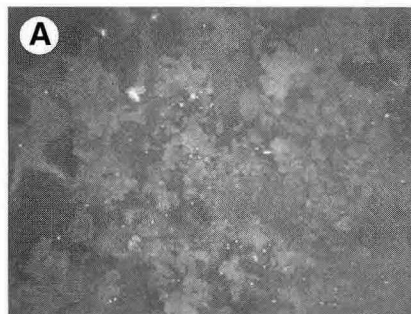


Figure 2. Induction of somatic embryo from the hypocotyl explant of *Daucus carota* L. A, Developing somatic embryo, Mature somatic embryo (arrow); B, Regeneration plantlets.

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