Expression of non-toxic mutant of *E. coli* heat-labile enterotoxin (LT) in transgenic tobacco chloroplasts

한소천, 강귀현, 김미영, 김영숙, 강태진[1], 양문식
전북대학교 생물과학부, [1]전북대학교 기초과학연구소
전화 (063) 270-3569, FAX (063) 270-4334

Abstract

Escherichia coli heat-labile toxin (LT) is a potent mucosal immunogen and immunoadjuvant towards co-administered antigens. We describe a novel LT mutant with greatly reduced toxicity that maintains most of the adjuvanticity. The non-toxic mutant (LTK63), that contains a substitution Ser → Lys in position 63 of A subunit. LTK63 gene was cloned into chloroplast targeting vector for high level expression and material inheritance. The recombinant vector was transformed to tobacco by particle bombardment. PCR and Southern blot analyses confirmed stable homologous recombination of the LTK63 gene into the chloroplast genome. Western blot analysis showed that the chloroplast-synthesized LTK63 assembled into oligomers and were antigenically identical with purified native LTB. GM1-ganglioside binding assay confirmed that chloroplast-synthesized LTK63 binds to the intestinal membrane GM1-ganglioside receptor. Successful expression of foreign genes in tobacco chloroplasts augurs well for development of vaccine in edible parts of transgenic plants. (This research was supported by a grant for international cooperation from the Ministry of Science and Technology, South Korea)

References

1. Clements J. D., Hartzog N. M., Lyon F. L. (1988), Adjuvant activity of *Escherichia coli* heat-labile enterotoxin and effect on the induction of oral tolerance in mice to unrelated protein antigens, *Vaccine* **6**, 269-277.
2. Daniell H, Lee S. B., Panchal T., Wiebe P. O. (2001), Expression of the native cholera toxin B subunit gene and assembly as functional oligomers in transgenic tobacco chloroplasts, *J. Mol. Biol.* **311**, 1001-1009.
3. Douce G., Turcotte C., Cropley I., Roberts M., Pizza M., Domenghini M., Rappuoli R., Dougan G. (1995), Mutants of Escherichia coli heat-labile toxin lacking ADP-ribosyltransferase activity act as nontoxic, mucosal adjuvants, *Proc. Natl. Acad. Sci. USA.* **92**, 1644-1648.