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Purification of Recombinant Soybean Cold-acclimation Proteins by Affinity Chromatography and the Enhancement of Freezing-stress and Salt-stress in *E.coli* cells

Kee-Young Kim, Seong-Whan Park, Seon-Young Chung, Hye-Jeong Lee, In-Su Kim,
Chang-Woo Cho, Jai-Heon Lee¹

Faculty of Natural Resources and Life Science, Dong-A University

Objectives

- For production of polyclonal antibody, we purify soybean cold-acclimation proteins which are overproduced in *Escherichia coli*.
- We investigate the protective function of the fusion proteins against freezing- and salt-stress in *E.coli* cells.

Materials and Methods

1. Materials - plant: glycine max cv. sinpaldal2, host cell: BL21 (DE3), vector: pET
2. Method - low temperature treatment(4°C), affinity chromatography, northern blot, freezing and thaw experiments, salt treatment, preparation of polyclonal antibodies, SDS-PAGE and immunoblotting

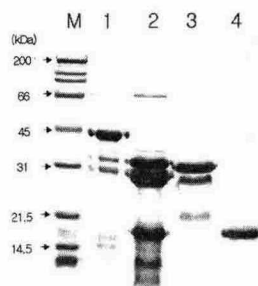


Figure 1. SDS-PAGE analysis of recombinant protein produced in *E.coli*, Lane 1, [ET-SLTI66 protein (41 kDa); Lane 2, pET-SLTI66 protein separated by Factor Xa; Lane 3, Factor Xa; Lane 4, SLTI66 protein (19 kDa)

Results and discussions

Maximal production of soluble cold-acclimation proteins was obtained by inducing expression of the cloned cold-acclimation genes with IPTG when the cells reached an OD₆₀₀ of 0.6-0.8. The recombinant proteins purified with a three-step purification scheme: fractionation, immobilized metal ion affinity chromatography, and gst affinity chromatography (Figure 1). Figure 2 shows the growth curves of *E.coli* cells by salt stress. In the 1% NaCl LB medium, there is no difference between the cell growth. In the 3% NaCl medium, much better cell growth level was observed in the *E.coli* cells with *SLTI66* gene compared with the control cells. In the 5% and 7% NaCl medium, they both showed only a slight increase in OD₆₀₀ value 10h after the inoculation of cells. This result indicates that the expression of the *SLTI66* gene has a protective function against the damage to the cells under salt stress.

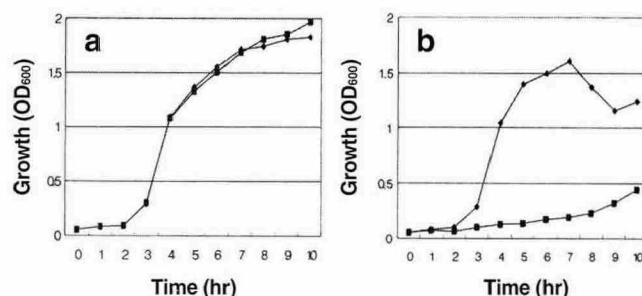


Figure 2. NaCl salt-stress tolerance of *E.coli* cells carrying *SLTI66* gene; (a) the growth curves of *E.coli* cells with *SLTI66*(◆) and pET 42a(■) in 1% NaCl medium, (b) the growth curves of *E. coli* cells with *SLTI66*(◆) and pET42a (■) in 3% NaCl medium.