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Enhancement of Freezing- and Salt-stress of *SLTI66*

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For production of polyclonal antibody, we purify soybean cold-acclimation proteins which are overproduced in *E. coli*. We investigate the protective function of the fusion proteins against freezing- and salt-stress in *E.coli* cells. 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 (fig 1). Fig 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.