Effect of silkworm hemolymph on protein expression and enzyme activity in *E. coli*

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Abstract

It was reported that silkworm hemolymph (SH) increases the host cell longevity by inhibiting baculovirus-induced insect cell apoptosis1 and also increases the production of recombinant protein in this system1,2. In this study, the effect of silkworm hemolymph on prokaryotic gene expression level and enzyme activity was investigated. E. coli BL21 (DE3) was used as a host cell and the production of β-galactosidase from the gene (lacZ) in host chromosome or pET22b was assayed. Supplementation with SH increased β-galactosidase activity; however, the results of SDS-PAGE and Western blotting showed that SH didn't affect the host and recombinant gene expression levels.

These results implicate that SH increases enzyme activity and/or stability but does not affect gene expression level. Moreover, the activity of β-galactosidase from cells lysed with a small quantity of toluene in the presence of SH showed as high as that from cells lysed by sonication. The β-galactosidase activity in Z buffer supplemented with SH increased 30% as compared with that of Z buffer alone. When SH was fractionated by gel-filtration chromatography, five fractions were obtained3. Among these fractions, FII and FIII showed a similar effect to the whole SH. SH also increased the stability of β?galactosidase. β?galactosidase lost its activity after 100 h incubation in Z buffer, while β?galactosidase showed still high activity in Z buffer supplemented with 5% SH.

1. Rhee W. J., Kim E. J., Park T. H. 1999. Kinetic effect of silkworm hemolymph on the delayed host cell death in an insect cell-baculovirus system. Biotechnol. Progress. 15: 1028-1032
2. Ha S. H., Park T. H. 1997. Efficient production of recombinant protein in Spodopterafrugiperda/ AcNPV system utilizing silkworm hemolymph. Biotechnology Letters. 19: 1087-1091
3. Kim E. J., Rhee W. J. Park T. H. 2001. Isolation and characterization of an apoptosis inhibiting component from the hemolymph of Bombyx mori. Biochemical and Biophysical Research Comunications. 285: 224-228