

High-Level Production of Spider Silk Protein by Fed-Batch Cultivation of Recombinant *Escherichia coli* and Its Purification

이석재, 이상엽

한국과학기술원 생명화학공학과 및 생물공정연구센터

전화 (042)869-3930, FAX (042)869-8800

Silk proteins from *Nephila clavipes* are fibrous proteins containing repetitive sequences with both crystalline and amorphous domains. In order to obtain high-level production of silk protein, the synthetic genes had 16 contiguous units of the consensus repeat sequence of the silk protein were expressed in *Escherichia coli* BL21(DE3) under the strong inducible T7 promoter. For production of recombinant silk protein in large amounts, pH-stat fed-batch cultures were carried out. The recombinant silk protein was produced as soluble forms in *E. coli*, and the recombinant silk protein content was as high as 11% of the total protein. When cells were induced at OD600 of 60, the amount of silk protein produced was 6.49 g/L. After simple purification steps, 9.2 mg of silk protein that was more than 80% pure was obtained from a 50 mL culture, and the recovery yield was 26.3%.

Acknowledgements

We thank Dr D. Kaplan for kindly providing the plasmid, pSH16A.

References

1. S. Winkler, S. Szela, P. Avtges, R. Valluzzi, D. A. Kirschner, D. Kaplan "Designing recombinant spider silk proteins to control assembly" (1999) *Int J. Bio. Macromol.* 24, 265-270.
2. H. Heslot "Artificial fibrous proteins: A review" (1998) *Biochimie*, 80, 19-31.
3. M. B. Hinman and R. V. Lewis "Isolation of a clone encoding a second dragline silk fibroin" (1992) *J. Biol. Chem.* 267, 19320-19324.
4. R. V. Lewis, M. Hinman, S. Kothakota and M. J. Fouriner "Expression and Purification of a spider silk protein: A New Strategy for producing repetitive proteins" (1996) *Pro. Exp. Puri.* 7, 400-406.
5. S. J. Park, G. Georgiou, and S. Y. Lee "Secretory Production of Recombinant Protein by a High Cell Density Culture of Protease Negative Mutant *Escherichia coli*" (1999) *Biotechnol. Prog.* 15, 164-167.