

Effect of rare codons on heterologous expression of proteins from the archaeon *Sulfolobus solfataricus* in *Escherichia coli*

Seonghun Kim^{1,2} and Sun Bok Lee^{1,2,3}

¹School of Environmental Science and Engineering, ²Division of Molecular Life and Sciences, and

³Department of Chemical Engineering, POSTECH, Pohang 790-784, Korea

TEL: +82-54-279-5970, FAX: +82-54-279-5528

Hyperthermophilic microorganisms are attractive sources for novel biocatalysts in that proteins purifications from these cell are often much easier than those from mesophilies^{1,2}. However, archaeal genes are usually poorly expressed in *Escherichia coli* because of the difference in codon usages³. The genes of thermoacidophilic archaeon *Sulfolobus solfataricus* have a high content of rare codons for arginine (*argU*), isoleucine (*ileW*), and leucine (*leuW*), which are rarely used in *E. coli*. To test rare codon effects, we heterologously expressed the five ORFs of *S. solfataricus* (SSO0981, SSO2493, SSO2585, SSO3107, and SSO3198) in *E. coli*. Unlike from other ORFs, SSO3198 was scarcely expressed when their gene was cloned in pRSET and expressed in *E. coli* BL21(DE3). However, the same gene could be successfully expressed in *E. coli* BL21(DE3)-CodonPlus RIL strain⁴, which contains extra copies of *argU*, *ileY*, and *leuW* tRNA genes. We tested the effects of the frequency of rare codons and locations of them on protein expressions of two ORFs, SSO3198 and SSO3107, which contain approximately the same rare codon frequency, by point mutation studies. From these studies, we found that heterologous expressions of SSO3198 in *E. coli* depended on the position of rarecodons rather than the frequency of rare codons.

References

1. Hough, D. W. and Danson, M. J. (1999) *Curr. Opin. Chem. Biol.* 3, 3946
2. Adam, M.W. W., Perler, F. B. and Kelly, R. M. (1995) *Biotechnology* 13, 66-668
3. Makrides, S. C. (1996) *Microbiol. Rev.* 60, 512-538
4. Carstens, C. P. and Waesche, A. (1999) *Strategies* 12, 49-51