**High level accumulation of soluble mutant L-asparaginase (Q59L) with co-expression of chaperones in *SHuffle*™*T7* strain**

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Introduction: There is an increasing demand for production of L-asparaginase in clinical researches and pharmaceutical industries. Q59L mutant of L-asparaginase has been recently introduced as a glutaminase-free enzyme with lower side effects in the treatment of leukemia patients. In the present study, we used an engineered *E. coli* expression strain, *SHuffle* *T7*, which helps to produce disulfide bond containing recombinant proteins in cytoplasm. Also the effect of chaperone molecules has been studied.

Methods: Recombinant pET28a- Q59LAsp construct, the commercial vector PG-Tf2 expressing molecular chaperone trigger factor and groELS chaperonin system has been cloned into *E. coli* expression strains. Recombinant Q59LAsp protein has been expressed in *BL21 DE3 and SHuffle T7* strainsandpurified using nickel affinity chromatography. Yield of soluble purified protein in each *E. coli* strain and in different conditions was determined and compared.

Result: The presence of chaperones enhanced the yield of soluble asparaginase enzyme in both *BL21 DE3 and SHuffle* *T7*strains. Also the new *SHuffle* *T7* strain has yielded more soluble asparaginase protein compared to *BL21 DE3* strain. The highest amount of soluble recombinant protein was obtained from *SHuffle* strains in the presence of chaperone.

Conclusion: According to the results of the current study, simultaneous use of new *E.coli* strains such as *SHuffle* with chaperones may be a suitable candidate in order to produce more amounts of the asparaginase mutant enzyme, having fewer side effects in treating patients with leukemia.

Keyword: Asparaginase, *E. coli*, Recombinant protein, *SHuffle*, Chaperone