Construction of new genetic tools as alternatives for protein overexpression in Escherichia coli and pseudomonas aeruginosa

ABSTRACT

Background: Pseudomonas protein expression in E. coli is known to be a setback due to signifi cant genetic variation and absence of several genetic elements in E. coli for regulation and activation of Pseudomonas proteins. Modifications in promoter/repressor system and shuttle plasmid maintenance have made the expression of stable and active Pseudomonas protein possible in both Pseudomonas sp. and E. coli. **Objectives:** Construction of shuttle expression vectors for regulation and overexpression of Pseudomonas proteins in Pseudomonas sp. and E. coli. Materials and Methods: Pseudomonas-Escherichia shuttle expression vectors, pCon2(3), pCon2(3)-Kan and pCon2(3)-Zeo as well as E. coli expression vectors of pCon4 and pCon5 were constructed from pUCP19-, pSS213-, pSTBlue-1- and pPICZαAbased vectors. Protein overexpression was measured using elastase strain K as passenger enzyme in elastinolytic activity assay. **Results:** The integration of two series of IPTG inducible expression cassettes in pCon2(3), pCon2(3)-Kan and pCon2(3)- Zeo, each carrying an E. coli lac-operon based promoter, Plac, and a tightly regulated T7(A1/O4/O3) promoter/repressor system was performed to facilitate overexpression study of the organic solvent-tolerant elastase strain K. These constructs have demonstrated an elastinolytic fold of as high as 1464.4 % in comparison to other published constructs. pCon4 and pCon5, on the other hand, are series of pCon2(3)-derived vectors harboring expression cassettes controlled by PT7(A1/O4/O3) promoter, which conferred tight regulation and repression of basal expression due to existence of respective double operator sites, O3 and O4, and lacIq. **Conclusions:** The constructs off ered remarkable assistance for overexpression of heterogeneous genes in Pseudomonas sp.and E. coli for downstream applications such as in industries and structural biology study.

Keyword: Elastase strain K; LacIq; Overexpression; Regulation; T7(A1/O4/O3)