Tight repression of elastase strain K overexpression by PT7 (A1/04/03) shuttle expression system

ABSTRACT

The PT7(A1/O4/O3) is a promoter resulted from construction of O3 and O4 operators into PA1, a promoter derived from coliphage T7, that evidenced lower the occupancy of the promoter by RNA polymerase and thereby increases the repression factor. A new expression system, pTEL, was successfully constructed via shuttle vector pUCP19 as the backbone as the former carries pre-existing stabilizing fragment (SF) that enables replication of the plasmid in both E. coli and Pseudomonas sp. The leaky lac operon-based promoter found in pUCP19 was subsequently replaced by the PT7 (A1/O4/O3). Meanwhile, structural gene of the organic solvent tolerant elastase strain K was used as DNA insert (passenger enzyme) for repression and overexpression studies. The success of pTEL was evidenced by detection of non-significant protein expression level in the absence of IPTG as the inducer, indicating tight regulation possessed by the modified promoter. The addition of IPTG, however, relieved repression and demonstrated overexpression of the elastase strain K in various strains of E. coli following several optimization studies.

Keyword: PT7 (A1/O4/O3); Repression; Over-expression; Organic solvent tolerant; Elastase strain K