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Review

Efficient genetic approaches for improvement of plasmid based expression of recombinant protein in *Escherichia coli*: A review

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ABSTRACT

During the past two decades, there has been an explosion of new knowledge and techniques in the field of recombinant protein expression. However, over-expression of "difficult to express proteins" with therapeutic importance continues to be a challenging task for successful commercialization of these proteins. With the emergence of the bio-similar market, enhancing the efficiencies of the production process has become a critical factor in the commercial viability of novel products. Despite the availability of numerous technological advancements, recombinant protein expression in *Escherichia coli* remains difficult. Therefore, addressing upstream bottlenecks in combination with genetically modified expression hosts could be a viable strategy to enhance production. Problems like poor expression, plasmid instability, protein aggregation, protein degradation, and metabolic stress associated with recombinant protein production need special consideration during bioprocess development at bioreactor level. However, a comprehensive universal strategy for attaining efficient expression in *E. coli* seems unrealistic and must be resolved empirically. In this review, we have discussed some common problems and their apparent solutions for plasmids based recombinant gene expression in *E. coli*.

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Contents

Abbreviations: *E. coli*, *Escherichia coli*; DsbC, disulphide bond C; ORI, origin of replication; SUMO, Small Ubiquitin-like modifier; PSK, post segregational killing; GST, glutathione S-transferase; ORT, operator-repressor titration; NusA, N-utilization substance A; T7 RNAP, T7 RNA Polymerase; Pfg27, Plasmodium falciparum protein; IPTG, ß-D-thioglactopyranoside; pelB, pectatelyase B of Erwinia carotovora CE; TIR, translation initiation region; phoA, alkaline phosphatase; SD, Shine-Dalgarno; TAT, twin-arginine translocation; PNPase, polynucleotide phosphorylase; SRP, signal recognition particle; RBS, ribosomal binding site; Psp A, phage shock protein; RFs, release factors; TIR, translation initiation region; csp, cold shock protein; TF, Trigger factor; Trx, thioredoxin.

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1. Introduction

Designing an efficient bioprocess strategy is essential for cost effective production of recombinant proteins for clinical and industrial applications. A large number of expression hosts such as bacteria, fungi, insect cells, mammalian cells, plant cells, transgenic animals and plants are employed for this purpose. However, *Escherichia coli* has been preferentially used for the production of nearly 30% of the approved recombinant therapeutics due to its well-studied genetics, rapid growth and ease of high cell density fermentation on inexpensive cultivation media [1–3]. But, several upstream bottlenecks like codon usage, mRNA stability, and promoter strength critically hamper the overexpression of many eukaryotic proteins in this system. The overall productivity is also compromised due to metabolic burden experienced by protein producing cells which is attributed to depletion of precursors like amino acids and ribosomes, increased energy demands and initiation of cellular stress responses i.e. heat shock like response, SOS response and stringent response [4].

The hyper-expression of some of the eukaryotic proteins in *E. coli* leads to the formation of inclusion bodies (IBs) due to higher transcription rates. The solubility of protein is favored by using slow expression rates, increasing availability of sufficient refolding mediators and creating oxidizing environment in cell. However, none of these strategies guarantee soluble protein, and hence these need to be empirically optimized.

Protein degradation is another big problem associated with recombinant protein production in *E. coli* which adversely affects both the quantity and quality of recombinant products. The accumulation of protein in a heterologous host is governed majorly by rates of two processes i.e. protein synthesis and protein degradation. Elevation of protein synthesis rates alone cannot promise improvement in protein yields if the rate of degradation for the protein is high. Proteolysis constitutes 3 percent of total enzymatic activities taking place in *E. coli* at any given time [5]. Moreover, heat shock like response triggered by recombinant protein synthesis enhances the protease activity by several folds [6]. However, these responses diverge with the protein's specific characteristics such as solubility, structure, amino acids composition [7–9]. Thus, the problem of proteolysis of recombinant products needs to be carefully addressed.

Targeting all these upstream challenges is expected to improve final expression yields and promise cost effective production.The global factors such as metabolic burden and cellular stress responses can be addressed by optimizing culture conditions and also by manipulating expression host using system engineering approach [10]. Thus the task of achieving high level protein expression demands an integrated approach involving optimization of diverse components at various stages of process development. Keeping this in view, the present review comprehensively summarizes various genetic approaches to address the problems of poor expression, low solubility and protein degradation associated with recombinant protein production. Moreover, a deeper understanding of major cellular metabolic changes associated with recombinant protein expression is also provided.

2. Factors having impact on gene transcription

The principal components of a typical plasmid vector having influence on transcription efficiency include origin of replication (*Ori*), promoter, antibiotic selection marker, and transcription terminators (Fig. 1).

2.1. *Origin of replication* (Ori)

A plasmid's copy number can be manipulated by making alteration in its replicon and even a single point mutation can change copy number drastically. Although high copy number of plasmids in cells ensures multiple copies of gene of interest but it does not always promise higher yield of protein. Furthermore, high copy number plasmid puts extra metabolic load on bacterial cells, which reduces growth rate and favor plasmid instability, thereby diminishing the overall protein yield [11]. High copy number plasmid can be beneficial only if low copy number of gene is limiting the final expression level. However, if the bottleneck is at translational level then high copy number will lead to larger population of mRNA transcripts without any consequent improvement in protein synthesis, thus putting additional metabolic burden on cells [12].The choice of *Ori* is crucial for co-expression of different types of plasmids in cell. The plasmids carrying same *Ori* sites are incompatible for existing in cell as they compete for the common metabolic machinery and create an unstable and unpredictable environment [13]. Common plasmids and their origin of replication are listed in Table 1.

2.2. *Selection marker*

An antibiotic resistance marker is integral part of a plasmid backbone required to favor the growth of protein producing

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