

Expression of Foreign Proteins in Escherichia coli

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Research Article

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Abstract

Background

Optimization of conditions for the recombinant production of proteins in a prokaryotic expression system is essential as the recombinant proteins impose a metabolic burden on cell's growth leading to low protein yield and low protein expression resulting from cell death.

Main text

The concentration of media components is optimized to accommodate for depleted nutrients due to foreign protein expression. The temperature is optimized to reduce proteolytic degradation and accumulation of protein as inclusion bodies in *Escherichia coli*. The concentration of inducer and time of induction for high protein yield is also optimized. These optimization conditions depend on the promoter under which the gene of interest is present and the characteristics of the target protein.

Conclusion

In the past few years, many optimization conditions for the production of recombinant proteins in *Escherichia coli* have been studied. These conditions depend mainly upon the promoter used to produce protein and the type of protein produced. Optimizing the expression parameters of protein produced in *Escherichia coli* ensures maximum yield of the desired protein.

Background

Proteins are essential components of life and constitute the majority of living organisms. They play crucial roles in a number of cell processes, including cell signalling, immune responses, cell adhesion, and cell cycle, and therefore their deficiency is associated with several disorders. With the advent of recombinant DNA technology in the late 1970s, proteins began to be produced in many host organisms, resulting in quicker and simpler processes relative to their natural sources [1]. With the advancements in biological therapeutics, the development of recombinant protein drugs on a wide scale is becoming increasingly essential. The optimizations of conditions for the expression of recombinant proteins have been carried out in a variety of expression systems like *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, insect cells, and mammalian cells [2–4]. Among these expression systems, the prokaryotic expression systems for the production of recombinant proteins are much preferred. *E. coli* was the first host in which recombinant DNA (rDNA) protein, insulin, was produced [5]. *E. coli* remains the dominant host for the production of recombinant proteins because it is inexpensive and cost-effective for mass production of recombinant proteins. *E. coli* has a fast growth rate with early protein expression and high protein yield, which makes it preferable over other expression systems. Because of the better understanding of *E. coli* mode of transcription and translation, it is easier to manipulate its genome to optimize protein expression.

A large number of commercially important proteins are recombinantly produced in *E. coli*, including T4 DNA ligase, CD4 and viral antigens for vaccines [6–8]. The expression of proteins in *E. coli* is achieved by inserting the gene for the recombinant protein in an expression vector under the control of a constitutive or regulatable promoter. Constitutive promoters are used for the overproduction of recombinant protein by continuous production, but they impart metabolic stress on cells energy resources leading to growth inhibition and ultimately lower yield of recombinant protein [9]. The expression of target proteins can be controlled by influencing various factors like temperature, growth media composition etc., under the control of regulatable promoters [10]. For example, the *lac* promoter, which is only induced in the presence of lactose or its derivative Isopropyl- β -D-ThioGalactopyranoside (IPTG) [11]. The ability to induce the expression of the foreign gene allows the cell growth time to be separated from induction time. Allowing the cells to grow at a certain level before induction results in a higher yield of target protein, and the protein expression conditions could be further improved by optimizing other parameters like the concentration of inducer, fermentation time etc.

The lactose (*lac*) promoter from *E. coli* is one of the most characterized promoters used extensively for the expression of recombinant proteins [12]. The wild-type *E. coli lac* operon consists of three genes (*lacZ*, *lacY* and *lacA*). They produce proteins involved in the metabolism of lactose. *lacZ* encodes β -galactosidase, which converts lactose to glucose and galactose, *lacY* helps transport lactose across the cytoplasmic membrane, *lacA* detoxifies harmful lactose analogues [13]. The *lac* repressor (product of *lacI*) regulated the transcription of recombinant proteins [14]. In the absence of an inducer, the *lac* repressor binds to the operator region of *lac* operon, preventing the RNA polymerase from binding on *lac* promoter region, and thus the *lac* genes are not transcribed. The *lac* repressor is not continuously bound to the *lac* operator because of the equilibrium that exists between the bound and unbound *lac* repressor molecules, so there is always a basal level transcription of *lac* genes [15]. When induced with lactose or its analogue IPTG, the transcription of the *lac* gene increases 1000 folds [16]. The basal level transcription of β -galactosidase converts lactose into allolactose which binds to *lac* repressor inducing a conformational change in it and hence allowing the transcription of *lac* genes.

The expression of recombinant proteins in *E. coli* imposes a metabolic burden on cell's growth leading to low yield of the target protein and retarded growth of culture [9]. The yield and expression of recombinant protein can be improved by optimizing the composition of growth media, the concentration of inducer, point of induction and fermentation time.

Main Text

The effect of IPTG concentration

The *lac* promoter is one of the most widely used promoters for the expression of recombinant proteins in the prokaryotic expression system. It is a regulatable promoter like mentioned previously, and it is induced by allolactose or its synthetic chemical analogue IPTG. IPTG binds to the *lac* repressor and induce a conformational change in it, disassociating it from the *lac* operator region and allowing RNA polymerase

to bind on the *lac* promoter region and synthesize the genes under *lac* promoter. Unlike lactose, IPTG is not restricted to by the *lac* permease, which makes it less susceptible to the inducer exclusion effect of glucose. IPTG is not metabolized within the cells; hence the concentration of IPTG stays the same throughout the experiment [17].

In literature, various IPTG concentrations ranging from 0.005mM to 1mM have been used to induce the expression of target protein but without any supporting data [18, 19, 17, 20]. Due to the metabolic burden on the cells, the high concentration of IPTG used to induce the expression of recombinant proteins thoroughly do not always lead to the maximum protein expression as IPTG induction can lead to an early onset of stationary phase [9]. In some studies, it was seen that the expression of target protein does not increase after a specific concentration of IPTG [21]. IPTG induction in a higher concentration may also induce many proteases leading to proteolytic degradation of recombinant protein. A recent study observed that a higher concentration of IPTG led to the accumulation of protein as inclusion bodies while lowering the concentration made the protein soluble [22]. The concentration of IPTG used to induce the expression of target protein depends on the genes under *lac* promoter, the quantity of *lac* repressor molecules and the target protein itself.

The effect of temperature

The temperature has a significant effect on the expression of the target protein in the prokaryotic expression system. The expression of the protein and the activity of the *lac* promoter is maximum at 37°C [23]. However, Optimization of temperature helps improve the final yield, expression and solubility of the target protein. At higher temperature, the target protein is more prone to proteolytic degradation and denaturation by heat, and thus the protein accumulates as inclusion bodies in *E. coli*. Lowering the temperature from 37°C to about 25°C has seen to reduce the proteolytic degradation of the protein and improve the stability of the target protein [24]. Lowering the culture temperature is also seen to enhance the proper export and folding of functional recombinant proteins [25]. As seen in the case of lower concentration of IPTG induction, lower temperature helps in the proper formation of recombinant proteins by reducing their overexpression which ultimately leads the protein to the folding pathways and hence protein aggregation does not occur [26].

The effect of media composition

The composition of growth media is seen to have a huge impact on the expression of the recombinant protein. The expression of protein under the *lac* promoter is optimized in simple and complex media in previous studies [27]. As mentioned previously, the expression of recombinant protein imposes a metabolic burden on bacterial culture. Complex media like the L.B and T.B media provides the culture with almost all necessary amino acids, vitamins and nutrients needed for growth; however, the complex nature of these mediums make it hard to point the limiting components on culture growth. Simple media provides the culture with a defined amount of amino acids and vitamins, and thus, they are mostly used in laboratories for the expression of recombinant proteins. Simple media is also less expensive compared to complex media, but the optimizations are necessary to achieve a high yield of the target protein [28].

Providing the growth media with peptone and the yeast extract significantly improves the expression of recombinant proteins [29]. In some cases where the amino acid composition of recombinant protein is different than the native proteins of the bacteria, the overexpression of target protein leads to nutrient depletion in the culture and early onset of stationary phase resulting in low protein yield and expression. The addition of transcription enhancers like cAMP in the media is seen to have a hugely positive effect on the expression of the target protein.

The effect of glucose concentration and lactose as an inducer

The presence of glucose in the culture during the induction phase is seen to have a negative impact on the expression of recombinant protein since glucose inhibits the expression of *lac* promoter via catabolic repression. A high level of glucose in culture results in a low level of cAMP, thus providing the culture with additional cAMP is seen to enhance the expression of recombinant protein (Fig. 1) [30]. Glucose is commonly used as a carbon source in media. Lowering the concentration of glucose below 0.1% w/v is seen to enhance the expression of the foreign protein [29].

Lactose is easily available commercially, but the use of lactose for induction of *lac* promoter over IPTG is very rarely reported in literature owing to the fact that lactose is naturally metabolized by bacterial cells, and its level do not stay constant throughout the experiment. Lactose is only allowed to enter the cells and induce the *lac* promoter when the glucose is depleted. Lactose is first converted to allolactose by β -galactosidases in order to induce the expression of *lac* genes; thus, it is important the bacteria have a basal level activity of β -galactosidases in order to use lactose as an inducer [31]. Lactose is very effective for the induction of *lac* promoter, just like IPTG. Unlike IPTG, the delayed response in protein expression is seen when induced with lactose because of the competition of other sugars with the same enzymes that activate lactose [32]. Lactose induction is sometimes preferred over IPTG because IPTG is toxic to humans, so it is important to have no traces of this chemical in the final product. Lactose also has an additional advantage of auto-induction when the media is refreshed with a sufficient amount of glucose and lactose and allowed to grow.

The effect of induction time and fermentation time duration

The time of induction and the duration for which the culture is allowed to grow are very crucial and must be optimized to ensure the maximum expression of the target protein. The bacterial growth period is divided into four phases (lag phase, log phase or exponential phase, stationary phase and the death phase). In the lag phase, the cells are just getting ready to divide, and the cells divide exponentially in the log phase. Due to the depletion of nutrients in the media, the cells go into a stationary phase and ultimately die. The time at which the culture is induced makes a huge impact on the expression of recombinant protein. Induction during the lag phase results in low protein expression because there is more media than the cells in culture and fewer cells to induce mean low protein expression. Induction during the log phase is most suitable for maximum protein expression because the cells are dividing rapidly, and there are more cells to induce. After passing the log phase, there is a huge risk of running into

dead cells in culture, which will not yield any protein [17]. When inducing with lactose, it is best to add lactose as soon as the glucose levels are depleted in culture.

The time duration for which the culture has proceeded after induction is important too. Sometimes the early expression of protein occurs, leading to the early onset of the stationary phase. However, it is reported that increasing the fermentation time leads to higher expression of the target protein [33]. It is important to observe the time at which the expression of the target protein is higher in the culture to make sure the protein is not degraded in culture due to temperature and proteases, and many other environmental factors. It also saves time by determining how long to proceed with a culture for maximum expression of the protein.

Conclusion

The recombinant production of protein in *Escherichia coli* has made it easier to produce biologically important proteins on an enormous scale. However, the production of protein in the *E. coli* expression system doesn't always yield maximum product because of various underlying parameters, which must be optimized depending on the promoter under which the protein of interest is inserted and the protein to be produced itself. This review article discussed some of the important parameters that are optimized for maximum expression of recombinant protein in *E. coli*. The expression of the protein is better when induced with IPTG instead of lactose because IPTG is not metabolized by bacterial cells during growth and remains constant throughout the process. The expression of the protein is observed to be high at temperatures of about 37°C. However, the temperature must be optimized according to the protein of interest since higher temperatures may lead to proteolytic degradation of the protein. Optimization of protein expression in *Escherichia coli* must be carried out experimentally to check what conditions best suit the recombinant protein.

Abbreviations

E. coli: *Escherichia coli*; recombinant DNA: rDNA; *lac*: lactose; IPTG: Isopropyl-β-D-ThioGalactopyranoside; L.B: Luria broth; T.B: Terrific broth

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

A systematic search was carried out from PubMed, Google Scholar and Google Web Browser by providing key terms "*E. coli* expression system, *lac* promoter, growth media effect, temperature effect, lactose and glucose concentration and fermentation period etc.". According to the particular contents, further literature was screened and analyzed. In this study, about 30 research articles were selected to make a comprehensive review.

Competing interests

The authors declare that they have no competing interests

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