COMPARATIVE STUDY ON CONVENTIONAL AND AUTO-INDUCTION FERMENTATION

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The biosynthesis of recombinant proteins with non-native structures and expressed in a non-soluble state is a major challenge. More than 30% of the recombinant protein produced by the most commonly used prokaryotic expression system (Escherichia coli) is produced in an insoluble form, further steps are required to solubilize it.

In our experiments, we compared the inducer effect, the monitored growth parameters of the conventional broth (LB, 2YT), and the auto-induction broth.

Keywords: auto-induction broth, recombinant protein, biosynthesis

List of abbreviations and definitions

IPTG - isopropyl-β-tipgalactoside
LB - Luria broth / Lennox broth / Luria–Bertani medium / Lysogeny broth
2YT - medium for the cultivation of *Escherichia coli*
ZYM - medium for auto-induction
ZYP - medium for auto-induction
pGEX-4T1 - pGEX-series GST fusion vector

1. Introduction

With the rapid development of recombinant DNA technology, it has become possible to introduce human genes into unicellular organisms and use its molecular toolbox to produce recombinant protein [1]. The first such cellular systems were bacterial bioreactors. The bacterial cell has proven to be an ideal organism for the production of many proteins and is still a widely used "protein" [2].

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The most commonly used is *Escherichia coli*, which together with the protein produced in the yeast system accounts for 40 percent of the production.[3,4] Bacterial bioreactors are very suitable for mass production, the production costs are relatively low and for the production of simple proteins that do not require secondary modifications, e.g. glycosylation (e.g., insulin, growth hormone) is the most appropriate system [5].

In most cases, the initiation of biosynthesis is accomplished by the addition of a conventional inducer, usually isopropyl-β-tipgalactoside (IPTG) [6]. The disadvantage of this method is that the growth of the bacterial culture must be monitored in a continuous way, and then, once the desired cell density has been reached, the inducing agent must be added. To overcome this drawback, auto-induction fermentation technologies have been developed. During the process, glucose, lactose, and glycerol are added to the medium used to grow the bacterial culture [7]. Glucose is the primary source of carbon for bacteria to grow, so cells use it to grow and maintain their life processes. Following the functioning of the lac system, when glucose is present in the nutrient solution, the cells use it and do not begin to utilize lactose. In the absence of glucose, cells begin to starve and, in the presence of lactose, transcription of the genes on the lac operon begins to utilize lactose, thereby also starting to express the target protein. Research has shown that cell density and the efficiency of protein production are much higher when another carbon and energy source is used in parallel with lactose. Suitable for this purpose is glycerol, which does not induce lactose catabolite repression, and is now a standard component providing a source of carbon and energy [8].

2. Materials and Methods

2.1. Chemicals and reagents

All chemicals used for experiments were purchased from commercial sources and are of analytical and molecular biology grade.

2.2. Recombinant plasmid and bacterial strains

E. coli strain BL21 STAR (DE3) *(F-ompT hsdSB (rB-, mB-) galdcmrne131)* was used as the host for protein expression. Plasmid pGEX-4T1 – which carries the tac promoter, a GST tag coding sequence, and ampicillin resistance gene – was used as an expression vector for GST protein (Fig. 1).

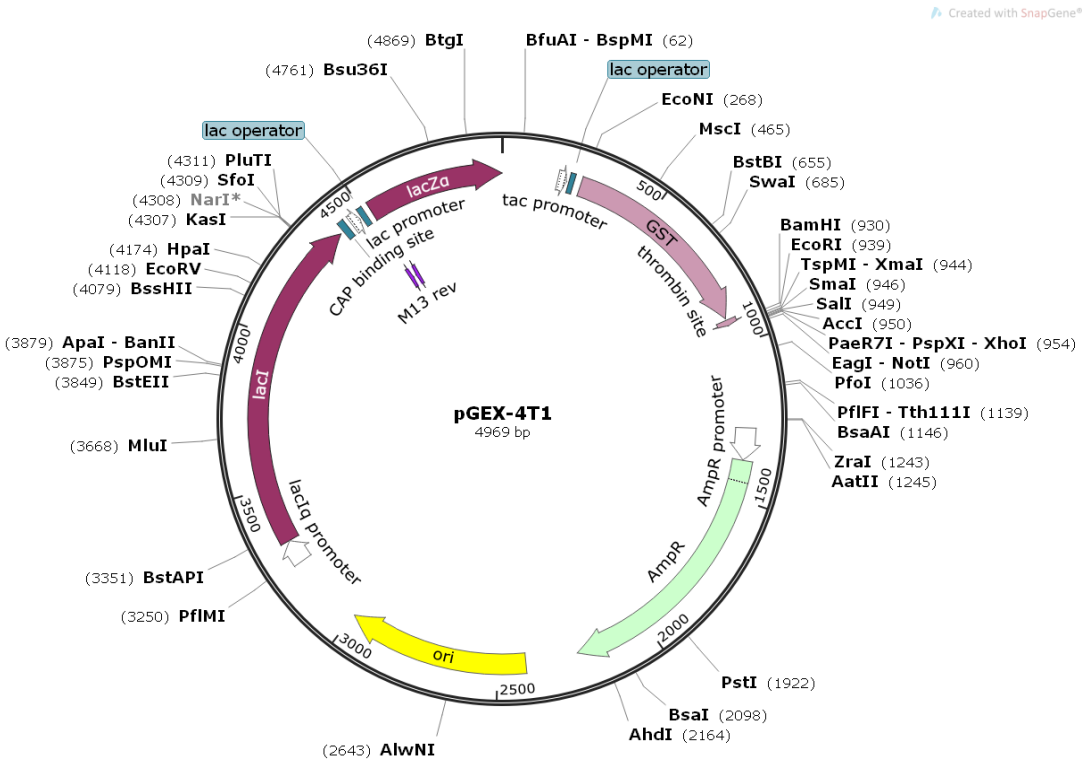

Fig. 1. pGEX-4T1 vector map

2.3. Bacterial growth curve analysis

Fresh cells of each strain were resuspended in the required medium to the initial A595 of 0.01. Wells in the microplate was filled with this suspension (200 µL in each well). The absorbance in each well was measured at 595 nm at 20 min with intensive shaking of the microplate. Data are shown either as the average of 4–8 parallel growth curves.

Calculations and graphs were performed with Microsoft Excel 2013 and MARS Data Analysis Software v.1.10.

3. Results and discussions

Fig. 2 shows the growth curve of the bacterial culture under different conditions: conventional LB and 2YT, IPTG induction broths, and ZYM and ZYP autoinduction broths. The notation "-" in the caption indicates the absence of an inducing agent (IPTG, lactose). The effect of the added inducing agent is examined in Fig. 3. In the conventional IPTG induction method, the inducer has a negative effect on the growth curve of the bacterial culture. The addition of the inducer delays the onset of the exponential phase, while also negatively affecting

the maximum growth rate. The presence of the inducer shows a similar trend for LB and 2YT medium. Unlike media using the traditional IPTG induction method, the autoinduction method does not show a negative effect of the inducing agent on the growth of the bacterial culture.

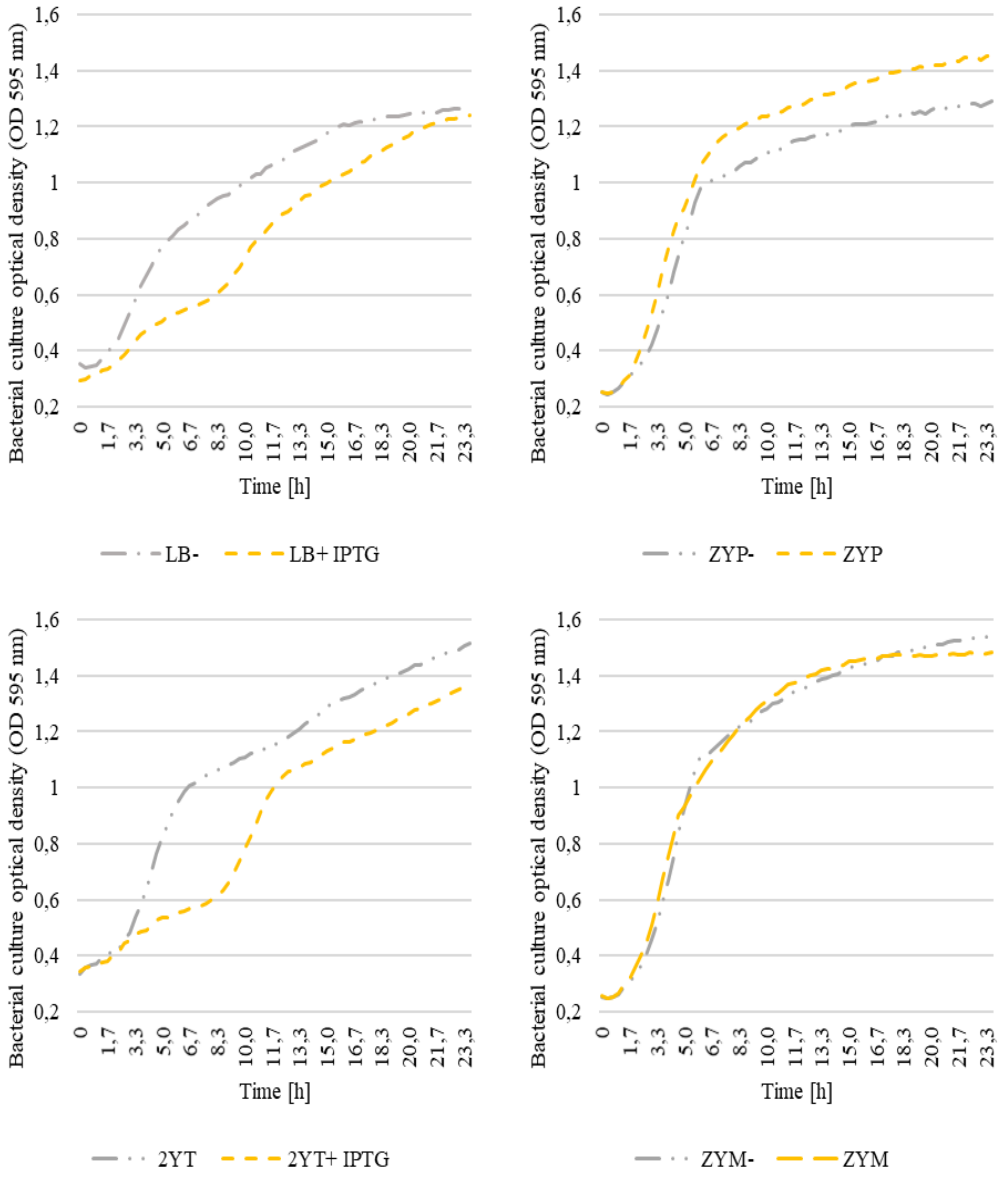

Fig. 2. Bacterial culture growth curves under different conditions: conventional LB and 2YT, IPTG induction broths, and ZYM and ZYP autoinduction broths. The notation "-" in the caption indicates the absence of an inducing agent (IPTG, lactose)

As shown in Fig. 3, a higher maximum growth rate can be achieved by using autoinduction broth. No negative effect of the inducer on either the bacterial growth curve or maximal growth was detected with ZYM and ZYP autoinduction broths. Fig. 3 shows the maximum growth rate of bacterial cultures tested under different conditions.

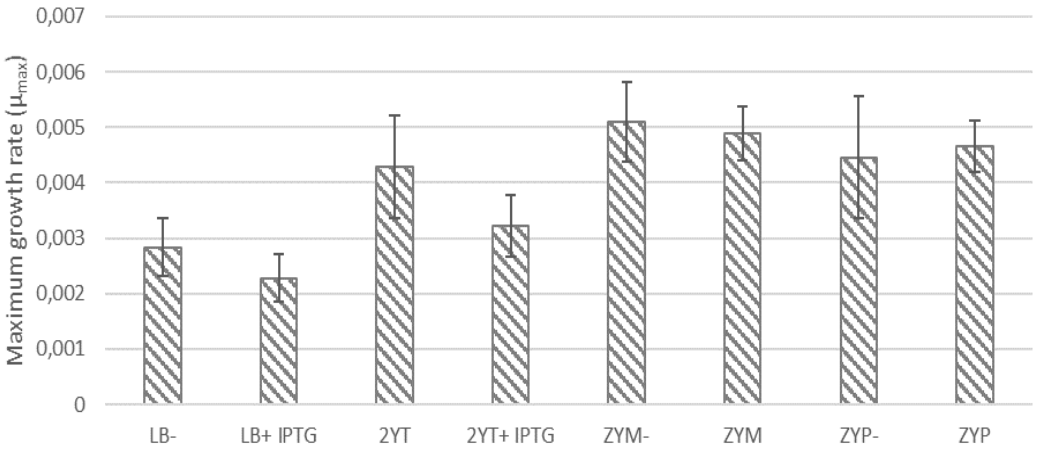

Fig. 3. The maximum growth rate of bacterial cultures

4. Conclusions

The production of multigram amounts of recombinant proteins requires an increase in the efficiency and scale of the production of the target protein. The most common method is IPTG-induced protein production in the LB medium. The disadvantage is that it is carcinogenic and expensive. By combining lactose with glucose, target protein production can be timed in *E. coli* culture (without external intervention). The usefulness of our research is to explore the effect of the inducing agent used in auto-induction fermentation on the growth curve and maximum growth rate of the culture.

In our experiments, we examined the inducer effect, the monitored growth parameters of the conventional broth (LB, 2YT), and the auto-induction broth. No negative effect of the inducer on either the bacterial growth curve or maximal growth was detected with ZYM and ZYP autoinduction broths. Unlike media using the traditional IPTG induction method, the autoinduction method does not show a negative effect of the inducing agent on the growth of the bacterial culture. In the conventional IPTG induction method, the inducer has a negative effect on the growth curve of the bacterial culture. The addition of the inducer delays the

onset of the exponential phase, while also negatively affecting the maximum growth rate.

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