Mongolian Journal of Biological Sciences 2003 Vol. 1(1): 77-80

Investigation of Growth Acceleration Factors of *E. coli ET2174* by Use of DO Signal

Batdorj Batjargal*, Mikio Nakajima and Toshiomi Yoshida****

**Department of Biochemistry and Bioorganic Chemistry, Faculty of Biology, National University of Mongolia, Ulaanbaatar 210646, Mongolia*

**International Center for Biotechnology, Osaka University 2-1, Yamada-oka Suita, Osaka 565-0871, Japan*

Abstract

Specific growth rate of *E. coli* AT2471 was estimated by on-line monitoring of DO level. The following results were obtained. Amino acid content of preculture medium was the sole reason for the two stages growth of recombinant strain *E. coli* AT2471. The experiment of on an individual amino acid influence showed that the addition of most acids contained in the preculture medium, except valine, cysteine and methionine, have neither beneficial nor negative effects on the cell growth. Valine stopped the cell growth and addition of isoleucine could reduce this negative effect. Addition of cysteine to the medium increased specific growth rate of cells from 0.49 h^{-1} to 0.62 h^{-1}; methionine addition increased it to 0.69 h^{-1}. The combination of these two amino acids enhanced cell growth resulting in a high value of μ 0.91 h^{-1}.

Key words: *E.coli* AT2471, two stages, growth, DO, specific growth rate, amino acids

Introduction

Microbial production of phenylalanine is known to be a process of low efficiency because of the considerable amount of energy required by the cells for phenylalanine synthesis (Hermann, 1987). To make the process profitable, special efforts have been focused on the improvement of this efficiency, which is usually expressed in terms of phenylalanine yield, absolute phenylalanine concentration and productivity. So far, it has been found that several factors affect phenylalanine synthesis, and their influences have been studied in the cases of different producents (Konstantinov, 1991). Phenylalanine synthesis is depends on various phenomena. As a result of several investigations the phenylalanine concentration, production rate and production yield based on the amount of glucose consumed were improved to 17g/l, 0.3g/l/h and 18%, respectively (Takagi, 1996). For high productivity it is essential to keep the specific growth rate of cells at the high rate during cultivation.

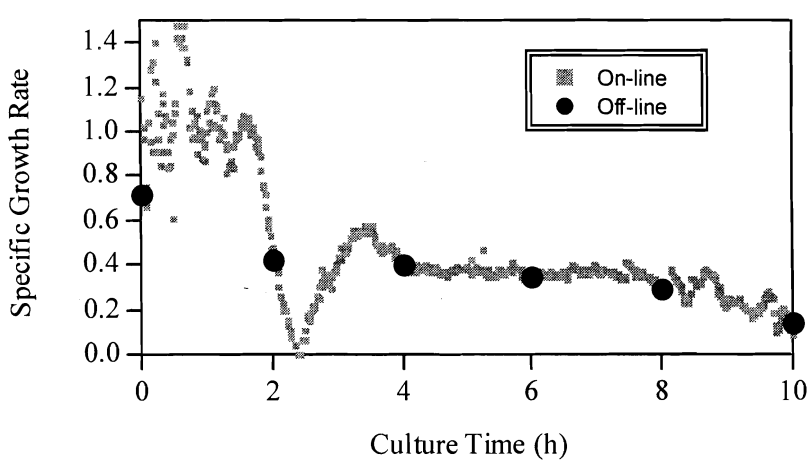

Fig. 1. Time course of specific growth rate of recombinant E. coli AT2471.
(On-line) Specific growth rate measured with on-line laser turbid sensor
(Off-line) Specific growth rate measured by using off-line sample

It was previously found in our laboratory that a recombinant strain *E. coli* showed two stages of growth: the fast growth in the beginning and slow growth in the latter phase (Figure 1).

Figure 1 shows that in the early stage of fermentation the cell growth rate is very high and reaching to the level of 1.0 h^{-1}. Suddenly cell stopped to grow and specific growth rate dropped to 0 after two and little more hours. Then cell restarted growing at a lower rate than the first growth. In this stage specific growth rate was around 0.4 h^{-1}. It was assumed that cells consume favourable substrate during the first growth and when this substrate exhausted cell growth stopped until bacteria establish a new metabolic system.

The specific growth rate was measured with on-line laser turbid sensor and by using off-line sample. Resolution of the off-line data does not allow us to distinguish these two stages of growth. On-line data using laser turbid sensor is adequate but not always applicable. Therefore, we have used a DO sensor for determination of the specific growth rate, using mass balance equation (Eq.6).

$$\frac{dX}{dt} = \mu X \qquad (1)$$

$$X = X_0 e^{\mu t} \qquad (2)$$

$$\frac{dC}{dt} = k_L a(C^* - C) - Q_{O_2} X \qquad (3)$$

$$\text{If } \frac{dC}{dt} = 0 \qquad (4)$$

$$\text{Then } C^* - C = \frac{Q_{O_2} X}{k_L a} \qquad (5)$$

The specific growth rate was decided by equation (6) using equation (2)

$$\ln(C^* - C) = \ln(\frac{Q_{O_2} X_0}{k_L a}) + \mu t \qquad (6)$$

When agitation rate is constant then $k_L a$ is also constant and initial cell concentration (X_0) is constant too. Only specific oxygen consumption rate (Q_{O2}) and specific growth rate (μ) are variables in Equation (6). The increase of specific oxygen consumption rate shifts the line (Eq.6) above, but when μ increases the slope of this line will increase. By measuring the slope of the line we can measure the specific growth rate.

The purpose of this study is to investigate the compounds that influenced on the growth of *E.coli* AT2471, using DO signal.

Materials and Methods

Bacterial strain. The tyrosine and thiamine-requiring mutant *E. coli* AT2471 was used as a host strain. It was transformed with the plasmid pSY130-14 carrying the genes *aro*F^{FR} and *phe* A^{FR} for enzymes involved in the critical steps in the phenylalanine synthesis (Sugimoto *et al.*, 1987). The plasmid also includes a gene for kanamycin resistance, and antibiotic was used to suppress the growth of plasmid-free cells.

Preculture. Stock culture was stored at –80°C in Luria-Bertani (LB) medium containing 20% glycerol and 25 mg/l kanamycin. For preculture 100 ml LB medium, which contained yeast extract (5 g /l); tryptone peptone (10 g/l); NaCl (10 g/l); kanamycin (25 mg/l) and antifoam (60 mg/l). One ml from the stock was inoculated and cultivated during14 hours in a 500 ml Sakaguchi flask at 26°C on a reciprocal shaker.

Cultivation. The main cultivation was carried out in a 5l jar fermentor. Initial volume was 3l. The cultivation medium had the following composition, as Na_2HPO_4 (7 g/l); KH_2PO_4 (4 g/l); K_2HPO_4 (4 g/l), NaCl (1 g/l); NH_4Cl (5 g/l); K_2SO_4 (2 g/l); $MgSO_4 \cdot 7H2O$ (3 g/l); trisodium citrate (10 g/l); sodium glutamate (0.55g/l); glucose (25 g/l); $CaCl_2$ (60 mg/l); thiamine (50 mg/l); $FeSO_4 \cdot 7H_2O$ (50 mg/l); tyrosine (600 mg/l); H_3BO_4 (0.5mg/l); $MnSO_4 \cdot 5H_2O$ (10 mg/l); $ZnSO_4 \cdot 7H_2O$ (3 mg/l); $AlCl_3 \cdot 6H_2O$ (3 mg/l); $NaMoO_4 \cdot 6H_2O$ (5 mg/l) and kanamycin (3 mg/l). The bioreactor was equipped with sensors for DO, pH and temperature. The following cultivation conditions were kept in the bioreactor unless otherwise mentioned: temperature 37.5°C; agitation rate 300 rpm; airflow rate 1vvm and pH 6.5.

On-line measurement. The following variables were monitored automatically: temperature, pH, DO, agitation speed and air flow rate. DO level of the main cultivation was monitored and recorded on-line. Specific growth rate was estimated by on-line monitoring of DO level using mass balance equation.

Results and Discussion

The recombinant strain of *E. coli* AT2471 shows two stages of growth during cultivation. We studied

experimentally to define what kind of substrate is contributed to these stages of growth. The first assumption was that cells may consume this substrate from the preculture medium and rapid growth is possible to occur when adding fresh preculture medium after the first growth to the cultivation medium.

Influence of preculture medium on the specific growth rate. Addition of fresh preculture medium after the first growth increased specific growth rate of cells from 0.49 h^{-1} to 1.12 h^{-1}. Figure 2 shows that preculture medium have a significant effect on the growth of recombinant strain *E. coli* AT2471. It suggests that cells consume a growth accelerating substrate from preculture medium. Previously this kind of phenomena on *E. coli* growth was explained by polyauxic growth on different carbon sources in the yeast extract by Rothen *et al.* (1998). In case of our strain we assumed that nitrogen source, especially amino acids have a significant growth effect.

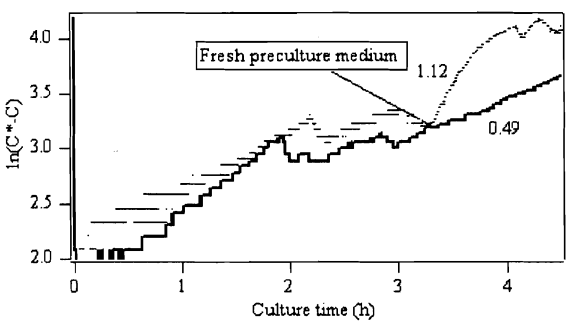

Fig. 2. Influence of preculture medium on cell growth.

Influence of all amino acid addition on the specific growth rate of cells. Special attention was given to amino acid utilization by the cells. The amino acid solution was prepared with the same composition of amino acids as in preculture medium by using the analysis of yeast extract and tryptone peptone and added to the cultivation medium after the first growth.

Addition of all amino acid combination is increased specific growth rate (μ) of cells from 0.49 h^{-1} to 1.06 h^{-1}. The growth acceleration was almost same as after adding fresh preculture medium (Fig. 3).

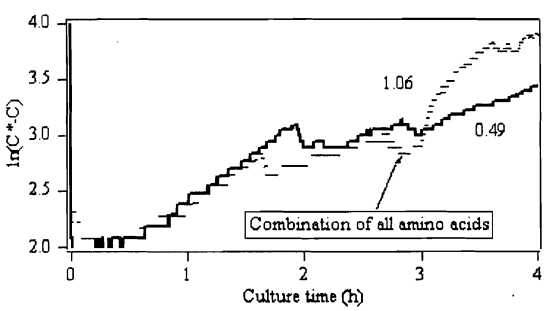

Fig. 3. Influence of amino acid combination on cell growth.

It suggested that amino acids of preculture medium contribute these two stages of growth. Pulse experiments were conducted to judge which amino acids enhance the growth rate of cells.

Individual addition of most amino acids contained in preculture medium, except valine, cysteine and methionine, had neither beneficial nor negative effects on the cell growth. Methionine and cysteine showed positive effects, while valine has a negative effect, and is stopped the cell growth. Further study shows that isoleucine could reduce negative effect of valine.

Effects of methionine and cysteine on the specific growth rate. Cysteine addition to the medium is increased growth rate of cells from 0.49 h^{-1} to 0.62 h^{-1}, while the addition of methionine is increased it to 0.69 h^{-1}. The combination of these two amino acids enhanced cell growth, resulting in a high value of growth rate, which is equal to 0.9 h^{-1} (Fig. 4).

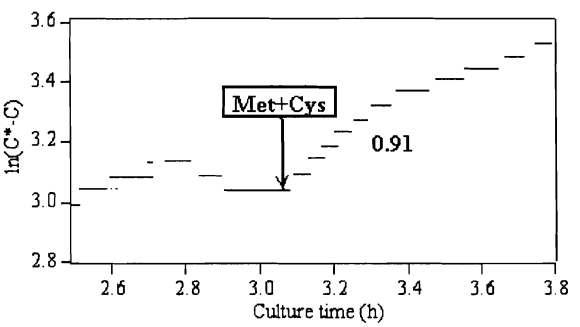

Fig. 4. Effect of methionine and cysteine combination on the cell growth.

Model of amino acid utilization. A model of amino acid utilization is developed on the basis of the results this study (Figure 5).

This model shows that valine and isoleucine have a common membrane carrier or an active pump. At the normal concentration rate of valine and isoleucine, active pump will work normally. If the concentration of valine is in excess, then

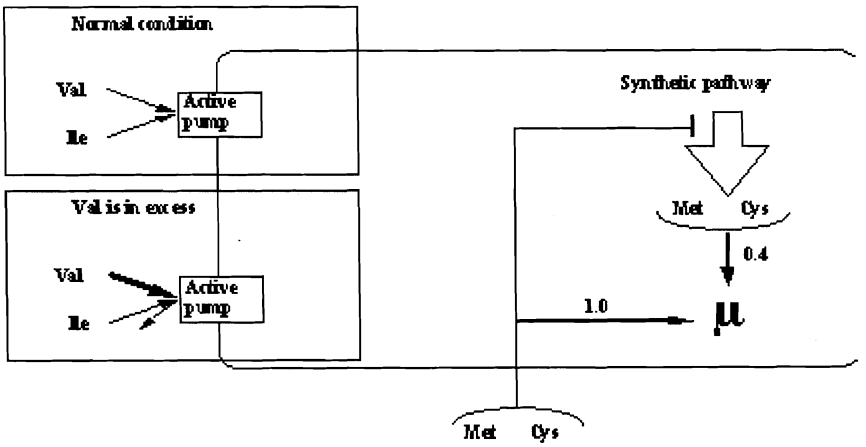

Figure 5. Model of amino acid utilization.

isoleucine could not enter into cell, and then cell growth stopped. However, further addition of isoleucine could recover this phenomenon.

When culture medium contains methionine and cysteine, cells consume these amino acids directly from culture medium, and the specific growth rate during this stage was equal to 1.0 h^{-1}. When these amino acids have exhausted, cells started to synthesize them by synthetic pathway. In this stage, specific growth rate of cells was equal to 0.4 h^{-1}.

References

Konstantinov K., Kishimoto M., Seki T. & Yoshida T. 1990. A balanced DO-stat and its application to the control of acetic acid excretion by recombinant *Escherichia coli. Biotechnol. Bioeng.* 36: 750-758.

Konstantinov K., Nishio N., Seki T. & Yoshida T. 1991. Physiologically motivated strategies for control of the fed-batch cultivation of recombinant *Escherichia coli* for phenylalanine production. *J. Ferment. Bioeng.* 71: 350-355.

Gschaeder A. & Boudrant J. 1994. Amino acid utilization during batch and continuous cultures of *Escherichia coli* on a semi-synthetic medium. *J. Biotechnol.* 37: 235-251.

Hermann K.M. & Somerwill R.L. 1983. Amino Acids: Biosynthesis and Genetic Regulation. Addison-Wesley, Massachusetts.

Takagi M., Yoshinori N., Gyuseop O. & Yoshida T. 1996. Control of L-phenylalanine production by dual feeding of glucose and L-tyrosine. *Biotechnol. Bioeng.* 52: 653-660.

Rothen A.S., Sauer M., Sonnleitner B. & Witholt B. 1998. Growth characteristics of *Escherichia coli* HB101 [pGEc47] on defined medium. *Biotechnol. Bioeng.* 58: 92-99.

(Accepted: December 2002)