Biotechnology Letters Vol II No 10 695-698 (1989)
Received as revised 2nd August.

PRODUCTION OF HUMAN LEUKOCYTE INTERFERON IN ESCHERICHIA COLI BY CONTROL OF GROWTH RATE IN FED-BATCH FERMENTATION.

Lee, Jae-Ho* , Youn-Hee Choi, Shin-Kwon Kang, Hyung-Hwan Park, and Ik-Boo Kwon

Lotte Group R&D Center, Biotechnology Section, C.P.O.Box 3013, Seoul, KOREA

SUMMARY: An E.coli harboring a vector containing double promoters, a signal sequence, and interferon gene was used. By fitting the feed rate of growth-limiting nutrients to the precalculated demand of the microorganism on the basis of a specific growth rate of 0.1 h^{-1}, fed-batch fermentations were performed. A cell density of 26 g/L was achieved after 46 hrs cultivation at 30°C. The culture was induced by IPTG and produced $1x10^9$ IU/L of human leukocyte interferon.

INTRODUCTION

The production of desired proteins by the use of recombinant DNA techniques has been largely centered upon the expression of heterologous genes in E.coli. To produce such proteins economically, high cell density culture methods are desirable when the production of end product is non-growth associated. However, there are special factors related to fermentations employing recombinant microorganisms(Zabriskie et al., 1986). Thus, excess concentration of glucose during aerobic yeast and E.coli fermentations leads to the production of ethanol and acetic acid respectively. There are also reports suggesting that the limitation of specific growth rate is important(Seo et al.,1985; Meyer et al.,1985). In this experiment, we tried to control specific growth rate for the production of end product by using the auxotrophic demands of the microorganism. At the same time, the effect of the vector containing a signal sequence on the localization of end product was investigated.

MATERIALS AND METHODS

Strain and plasmid: The host strain was E.coli JE5505(F^-, lpp-2, pps, his, proA, argE, thi, gal, lac, xyl, mtl, tsx). pIF-III-B encoding human leukocyte interferon directed by the lipoprotein and the lac double promoters was a derivative of pIN-III-B(Masui et al., 1983).

DNA manipulations: DNA manipulations were generally carried out following the procedures described by Maniatis et al.(1982).

Media: Semi-synthetic medium(KH_2PO_4, 3.5; $Na_2HPO_4.12H_2O$, 8.82;

K_2HPO_4, 2.0; $MgSO_4.7H_2O$, 1.33; glucose, 3.0; casamino acid, 3.0(in gram per liter); thiamine, 25 ug/ml; trace elements, 3ml/L; ampicillin, 50 ug/ml) was used. A solution of trace elements contained in gram per liter: $CaCl_2.2H_2O$, 0.75; $FeSO_4.7H_2O$, 27.78; $ZnCl_2$, 1.31; $CoCl_2.6H_2O$, 2.00; $CuCl_2$, 1.00; $Na_2MoO_4.2H_2O$, 2.00; H_3BO_3, 0.5; HCl, 100ml/L. Feeding solution consisted of 15%(w/v) glucose and 25%(w/v) casamino acid.

Cultivation: Fed-batch fermentations were carried out in 2L bench top fermentor(New Brunswick Scientific Co.,USA) with 1L initial working volume.The pH of the medium was controlled at pH 7.0 with 5N NaOH or 5N HCl using an automatic pH controller(Toyo Co.,Japan). Dissolved oxygen was maintained up to 10% by agitation speed(600-1,000rpm) and aeration (0.5-1.0 v.v.m.). 200ml of overnight culture was added to 1L of semi-synthetic medium and the fermentation was started at 30°C. As the cell density increased, the feed rate of feeding solution was increased to maintain an approximately constant specific growth rate. Phosphate and magnesium salts were added to the medium intermittently.Expression of lac promoter was induced by IPTG.

Analytical methods: Glucose was assayed by Somogy-Nelson method(Nelson, 1944). Recovery of extracellular, periplasmic, and intracellular proteins was following the method of Cornelis et al.(1982). Interferon activity was determined by measuring the inhibition of the cytopathic effect of vesicular stomatitis virus(VSV) on Hep2 cells.The titers were given in International Research Units. Dry cell weight was measured after drying the cells at 105°C overnight, and optical density was determined at 660nm. Plasmid stability was measured by replica plating from non-selective agar plates to selective agar plates. The ratio of the number of colony forming units on the selective agar plates to those on the non-selective agar plates was used to provide a percentage of cells containing the plasmid.

RESULTS AND DISCUSSION

It is known that recombinant proteins accumulate to higher concentrations when they are expressed intracellularly than when secreted(Marston et al., 1987). However, many protein products located in the cytoplasm have a tendency to aggregate, and specific solubilization techniques are often required. On the other hand,secretion from E.coli overcomes insolubility and facilitates purification, but further development is still required to improve yields(Marston et al., 1987). In this experiment, we used pIN-III-B vector which had a lipoprotein promoter of E.coli, a lac promoter, a lac operator and a lipoprotein signal sequence in tandem. Interferon gene was inserted into Hind III and BamHI sites at the end of lpp signal sequence(Figure 1). Some of the produced interferon was then enabled to be secreted into culture medium even when expression of the lac promoter was triggered.
Acetic acid is produced in E.coli when the specific growth rate or concentration of glucose is high(Zabriskie et al., 1986). As the employing strain, JE 5505, had auxotrophic markers, casamino acid was to be used as the growth-limiting

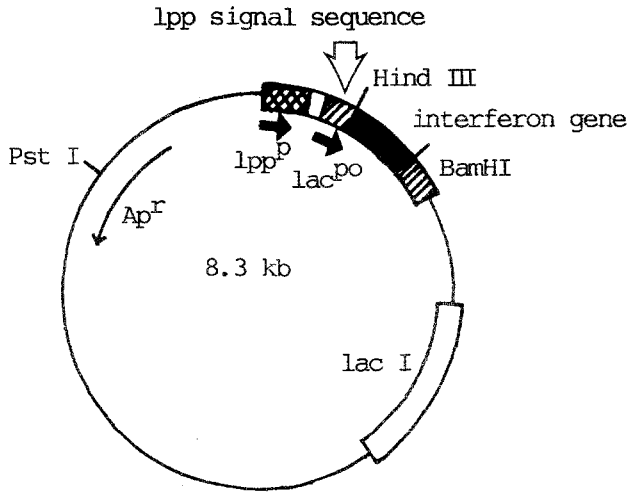

Figure 1. Schematic diagram of pIF-III-B.

nutrient; the Ks value of casamino acid in the Monod equation is larger than that of glucose, it was desirable that casamino acid was used for the growth-limiting nutrient while the supply of glucose was limited to minimum amount in order not to cause the glucose effect. Based on a specific growth rate of $0.1 h^{-1}$, culture time was divided by appropriate steps and the rate of feeding solution was changed to supply the precalculated required amounts of nutrients according to each step(Wang et al., 1979). By this policy, we could obtain a dry cell weight of 26 g/L after 46 hrs at 30°C(Figure 2). From start point to 34 hrs cultivation, no significant amount of acetic acid was detected and only 6ml of 5N NaOH was consumed to adjust the pH-value to 7.0. Average specific growth rate was $0.075 h^{-1}$ and overall growth yield about 0.34g cells/g glucose and 0.21g

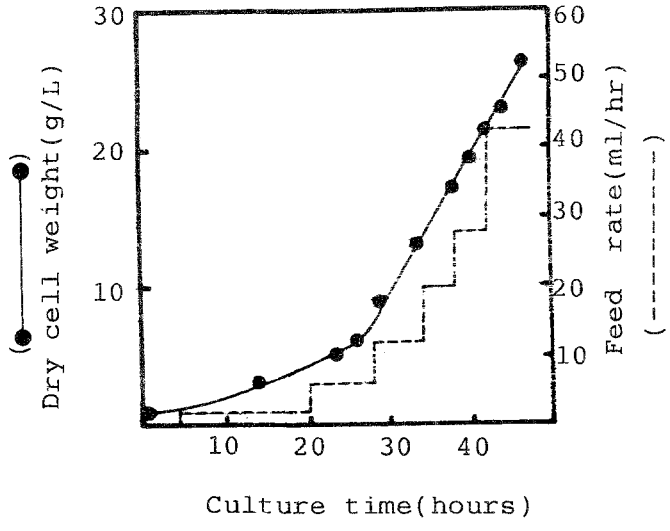

Figure 2. Trends of dry cell weight and the feed rate of feeding solution.

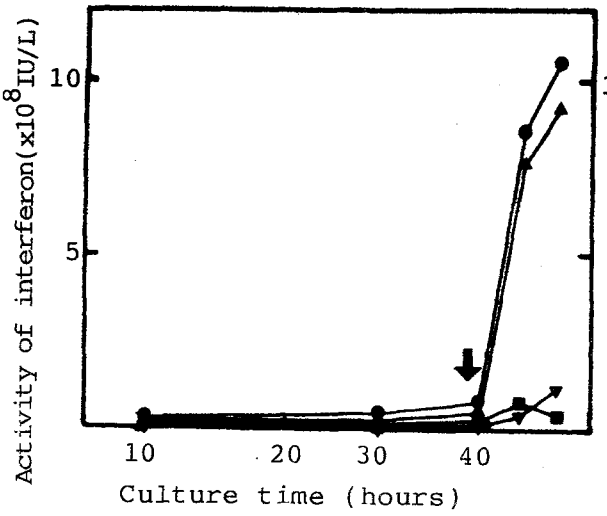

Figure 3. Localization of activity of interferon. Arrow indicates the time of induction.
●——● ; total activity,
▲——▲ ; periplasmic activity,
▼——▼ ; extracellular activity,
■——■ ; intracellular activity.

cells/g casamino acid. After 39 hrs, expression of lac promoter was induced by IPTG(final 1mM) and interferon was rapidly produced to $1x10^9$ IU/L(Figure 3). Most of the produced interferon was located in the periplasmic space of JE 5505 as expected. Throughout the experiment, concentration of glucose in medium was maintained below 1g/L and problems of plasmid stability were not observed.

ACKNOWLEDGEMENTS

We thank Mr. Y.K.Lee for his kind advice for this experiment.

REFERENCES

Cornelis, P.,Digneffe,C.,and Willemot,K.(1982).Mol.Gen.Genet., 186, 507-511.

Maniatis,T.,Fritsch,E.F., and Sambrook,J.(1982). Molecular Cloning, A Laboratory Manual, New York, Cold Spring Harbor Laboratory.

Marston,F.A.O.(1987). The purification of eukaryotic polypeptides expressed in E.coli. In: DNA cloning, D.M.Glover,ed. vol.3,pp59-87, IRL Press.

Masui,Y.,Coleman,J.,and Inouye,M.(1983).Multipurpose expression cloning vehicles in E.coli. In: Experimental manipulation of gene expression, Inouye,M.,ed.pp15-31, Academic Press.

Meyer,H.-P.,and Fiechter,A.(1985). Applied Environ. Microbiol. 50,503-507.

Nelson,M.J. (1944).J.Biol.Chem.,124,1375.

Wang,H.Y.,Cooney,C.L.,and Daniel,I.(1979). Biotech. Bioeng.,21, 975-995.

Zabriskie,D.W., and Arcuri,E.J.(1986). Enzyme Microb.Technol., 8,706-716.