

Full Length Research Paper

High level production of the recombinant gag24 protein from HIV-1 IN *Escherichia coli*

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The gag24 gene of the Human Immunodeficiency Virus type 1 (HIV-1) was expressed under the control of the tryptophan promoter in *Escherichia coli*. The effect of several parameters on the production of gag24 was studied. The expression level achieved (25%) depended on the host strain and the induction conditions. The developed fermentative process was scaled-up to 50 L, where 417 mg/L of volumetric production and 34 mg/L.h of productivity, were obtained.

Key words: *Escherichia coli*, gag24, HIV-1, scale-up.

INTRODUCTION

Escherichia coli is the most widely used prokaryotic system for the synthesis of heterologous protein due to its well-documented genetics and physiology (Panda, 2003). Achieving high expression levels of target protein is one of the main objectives in the cultivation of recombinant cells. This objective may be accomplished through two complementary approaches, namely, genetic engineering and optimal induction.

Different ways of inducing the expression of heterologous proteins cloned in *E. coli* under the ptrp system have been reported in the specialized literature (Yanofsky, 2003; Yanofsky, 2004). The most commonly used method is the use of competitive agents analogous to tryptophan, such as of β - indoleacrylic acid (IA), while the ptrp promoter can also be induced by derepression when the tryptophan present in the culture medium is depleted during the fermentation process (Bass and Yansura, 2000).

In this article, we report the effect of the *E. coli* host strain, and the induction conditions (IA and tryptophan concentration) in the production of the recombinant gag24 protein, expressed under the control of tryptophan promoter. Finally the fermentation process developed to shake flasks level was scale up to pilot plant level. We

describe a simple and highly efficient method for obtaining large quantities of gag24, which can be used for the development of HIV vaccine and for use in HIV diagnostic procedures.

MATERIAL AND METHODS

E. coli host strains and expression plasmid

The following *E. coli* strains were used: HB-101 and W-3110 (Sambrook et al., 2001). Bacteria were transformed with the pHIVCA-1 plasmid, which codes for the gag24 protein of HIV-1 under the control of the tryptophan promoter (Novoa et al., 1994).

Media composition and culture conditions

Cultures were performed in shake flasks (G-25, New Brunswick Scientific Co.) or in a B.E. Marubishi fermenter (Japan), using M9 medium (Miller, 1972) supplemented with glucose (5 g/L), casein hydrolysate (10 g/L), and 50 μ g/mL of ampicillin. This formulation will be denominated hereafter basal medium (BM). The culture parameters were: 37°C and 250 rpm in shake flasks, and 37°C, aeration rate 1 vvm, pH 7.0, and stirrer speed 350 rpm, in the 50 L fermenter. In all cases the induction time was 12 h. The influence of host strain was determined using BM supplemented with 10 μ g/mL of tryptophan. After 1 h of culture, 20 μ g/mL IA was added as inductor. The effect of IA was determined by adding 20 μ g/mL of tryptophan to the culture medium, and plus different concentrations of IA (between 0 to 60 μ g/mL) added after 1 h of cultivation. The influence of the initial tryptophan concentrations (from 0 to 100 μ g/mL) on protein expression was studied in the absence of IA in the culture medium. Finally the fermentation process developed to shake flasks level was scale up to pilot plant level using the kLa method.

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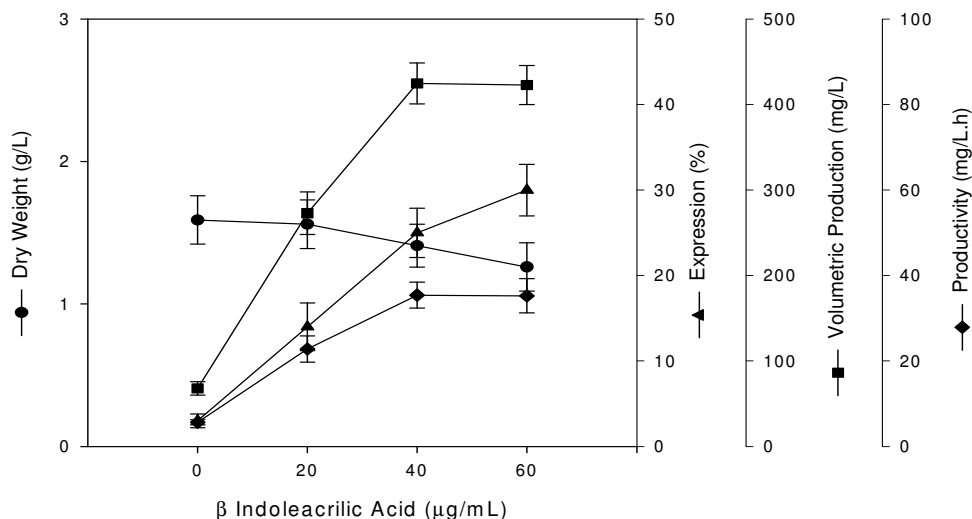


Figure 1. Effect of the IA concentration on the production of gag24. HB-101 (pHIVCA-1) transformed *E. coli* cells were grown in shake flasks using BM supplemented with 20 $\mu\text{g/mL}$ of tryptophan. After 1 h of culture, IA was added as inducer for achieving concentrations ranging from 0 to 60 $\mu\text{g/mL}$. The cells were cultured for 12 h at 37°C and 250 rpm.

Table 1. Effect of host strain on the production of gag24.

Host strain	Dry weight (g/L)	Expression Level (%)
HB-101	1.44 \pm 0.12	20 \pm 2
W-3110	1.47 \pm 0.18	15 \pm 3

Analytical methods

Cellular mass was reported as dry weight (g/L). The glucose was determined by the DNSA method (Sumner and Somers, 1949). In all cases the expression level (concerning the total cellular protein) was carried out by densitometric analysis of SDS-PAGE (Laemmli, 1970). The values of gag24 reported were determined multiplying the total protein concentration present in the culture medium (determined by the Lowry method (Lowry et al., 1951)) and the expression level. Each value corresponds to the average of at least 3 independent production batches, and in each experiment at least 3 assays were performed.

RESULTS AND DISCUSSION

Selection of the host strain

In order to assess the effect of the host strain in the expression of gag24, different strains were transformed with the pHIVCA-1 plasmid and grown in shake flasks using BM supplemented with 10 $\mu\text{g/mL}$ of tryptophan. 20 $\mu\text{g/mL}$ IA were added as inducer of the tryptophan expression, after 1 h of culture.

Table 1 shows the strong host strain dependence of protein expression level, while the cellular growth was very similar in both cases. Similar results had been previously reported by our group when studying the

expression of p36 protein from HIV-2 (Narciandi and Delgado, 1992), and a gene that codes for a fragment of the gp-41 transmembrane protein of HIV-1 (Narciandi et al., 1993), under the control of the tryptophan promoter. In the case of gag24, the highest yield was obtained using HB-101 (20% of the total cell protein).

The effect of the induction conditions on the production of gag24

The behavior of the expression of recombinant proteins expressed under the control of the tryptophan promoter depends on a series of factors, fundamentally the conditions of induction (Narciandi, 1996; Bass and Yansura, 2000).

Effect of IA concentration

The cells were grown in shake flasks. The BM was supplemented with 20 $\mu\text{g/mL}$ of tryptophan. After 1 h of culture, concentrations of IA (between 0 to 60 $\mu\text{g/mL}$) were added as inducer, and the incubation continued during 12 h at 37°C and 250 rpm. Figure 1 shows the effect of inducer concentration on the production of the gag24 recombinant protein expressed under the control of tryptophan promoter. The expression level, the productivity and the volumetric production are a function of the amount of inducer utilized. Using 60 $\mu\text{g/mL}$ of IA, 30% of expression was obtained, while using more than 40 $\mu\text{g/mL}$ of IA, the productivity and the volumetric production reach their maximum value (35 mg/L.h and 423 mg/L respectively). The use of high concentrations of IA is limited

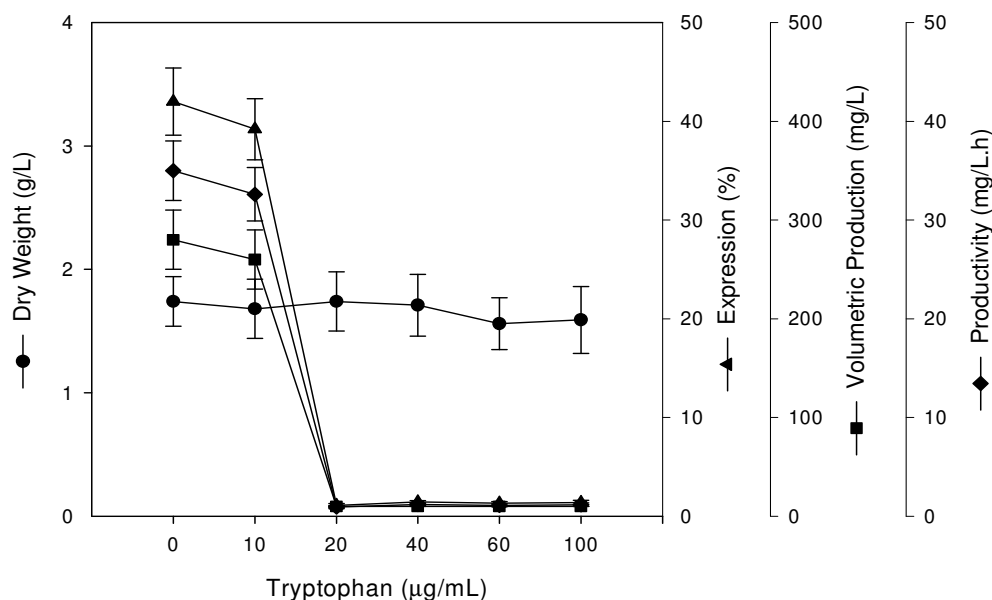


Figure 2. Effect of the tryptophan concentration on the production of gag24. HB-101 (pHIVCA-1) transformed *E. coli* cells were grown in shake flasks using BM supplemented with tryptophan concentrations ranging from 0 to 100 µg/mL in the absence of IA. The induction of gag24 expression was carried out by depleting the tryptophan present in the initial medium. The cells were cultured for 12 h at 37°C and 250 rpm.

to productive level due to their high cost. The cellular growth decreased only lightly upon increasing the concentration of the IA from 0 to 60 µg/mL. These results do not correspond with those reported in the literature, where concentrations higher than 25 µg/mL decreases drastically the cellular growth, the productivity and the expression of the recombinant proteins (Calcott et al., 1988).

When the gag24 production was evaluated in the absence of IA, a maximum dry weight of 1.59 was obtained, and the other analyzed parameters decreased significantly in comparison with values reported when the inducer was used. This can be explained by the inhibiting effects of the initial amount of tryptophan in the culture medium (20 µg/mL) (Shimizu et al., 1991).

Effect of tryptophan concentration

The expression of gag24 was studied using initial culture medium tryptophan concentrations that ranged from 0 to 100 µg/mL, and in the absence of IA. Figure 2 shows that the percentage of expression of the protein of interest decreased only slightly as the initial concentration of the repressor in the BM was increased from 0 to 10 µg/mL. However, concentrations above this level had inhibiting effects (Narciandi and Delgado, 1992; Pulliam et al.,

1997). Similar results were obtained when analyzing the productivity and the volumetric production of gag24.

The best yields (420 mg/L of volumetric production, 35 mg/L.h of productivity and 28% of expression) were obtained in absence of tryptophan, which is analogous to earlier report (Pulliam et al., 1997). Finally, cell mass was not noticeably affected in the presence of different tryptophan concentrations. These results are similar to the obtained using high concentrations of IA as inducer of the expression system. For this reason, the production of the gag24 protein was made by derepression of the expression system, without tryptophan in the culture medium.

Production kinetics of gag24

Scaling up of the HB-101 (pHIVCA-1) culture was carried out at 50 L level, using BM in absence of tryptophan. Data from a typical batch culture of *E. coli* producing gag24 are shown in Figure 3. The cell dry weight and the expression level increased gradually during the time of cultivation (12 h), to a final value of 3.9 g/L and 25%, respectively. The specific growth rate in the logarithmic phase was 0.92 h⁻¹. The productivity and the volumetric production had maximum yields after 12 h of culture (34 mg/L.h, and 417 mg/L, respectively). This corresponds with the decrease of the carbon source (glucose) in the

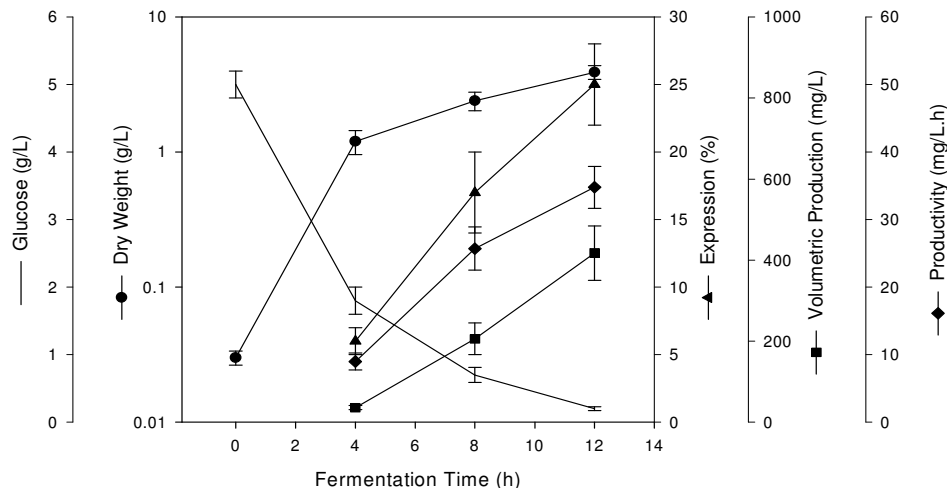


Figure 3. Kinetics of cell growth, product formation and substrate consumption. Scaling up of the HB-101 (pHIVCA-1) culture was carried out at 50 L level, using BM in absence of tryptophan. The induction of gag24 expression was carried out by exhausting the tryptophan present in the initial medium. The cells were cultured for 12 h at 37°C, aeration rate 1 vvm, pH 7.0, and stirrer speed 350 rpm.

medium. Similar results were obtained when the gag24 protein was expressed in shake flasks, indicating that the scale up of the fermentative process was effective. These results are several fold better than those reported previously for the expression and production of gag24 (Mills and Jones, 1990; Tanaka et al., 1992; Marcinovits et al., 1993; Hausdorf et al., 1994 ; Gupta et al., 2000; Zhang et al., 2002).

CONCLUSION

In this report we demonstrate that a high yield in the production of gag24 recombinant protein expressed under the control of tryptophan promoter, depends on the host strains, and the tryptophan and IA concentrations used in the culture medium. The fermentation process developed was scaled up satisfactorily to pilot plant level. We describe a simple and highly efficient method for obtaining large quantities of gag24, which can be used for the development of HIV vaccine and for use in HIV diagnostic procedures.

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REFERENCES

Bass SH, Yansura DG (2000). Application of the *E.coli* trp promoter. *Mol. Biotechnol.* 16: 253-260.

- Calcott PH, Kane JF, Krivi GG, Bogosian G (1988). Parameters affecting production of bovine somatotropin in *E. coli* fermentations. *Develop. Ind. Microbiol.* 29: 257-266.
- Gupta S, Arora K, Gupta A, Chaudhary K (2000). Gag-derived proteins of HIV-1 isolates from Indian patients: cloning, expression, and purification of p24 of B- and C-subtypes. *Protein Expr. Purif.* 19: 321-328.
- Hausdorf G, Gewie BA, Wray V, Porstmann T (1994). A recombinant human immunodeficiency virus type-1 capsid protein (rp24): Its expression, purification and physico-chemical characterization. *J. Virol. Methods* 50: 1-10.
- Laemmli UK (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- Marcinovits I, Boros I, Jarrah FE, Fust G, Molnár J (1993). Expression in *Escherichia coli* and in vitro processing of HIV-1 p24 fusion protein. *J. Biotechnol.* 31: 225-232.
- Miller JH (1972). *Experiments in molecular genetics*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: 431-432.
- Mills HR, Jones IM (1990). Expression and purification of p24, the core protein of HIV, using a baculovirus-insect cell expression system. *AIDS* 4: 1125-1131.
- Narciandi RE, García J, Motolongo J, Machado JA, Benitez JV, Novoa LI, Herrera L (1993). Production, isolation and semi-purification of the transmembrane gp-41 protein synthesized in *Escherichia coli*. *Biotechnologia Aplicada*. 10: 36-40.
- Narciandi RE (1996). High-Level Production of p36 from HIV-2 in Fed-Batch Culture of Recombinant *Escherichia coli*. *J. Ferment. Bioengin.* 81: 360-362.
- Narciandi RE, Delgado A (1992). Production of the recombinant p36 protein from HIV-2 in *Escherichia coli* using the trp promoter. *Biotechnol. Lett.* 14: 1103-1108.
- Novoa LI, Machado JA, Fernandez JR, Benitez JV, Narciandi RE, Rodriguez JL, Estrada MP, Garcia J, Herrera L (1994). Method for the expression of heterologous proteins produced in fused form in *E. coli*, use thereof, expression vectors and recombinant strains. *European Patent Application*, no. 90202108.8.
- Panda AK (2003). Bioprocessing of therapeutic proteins from the inclusion bodies of *Escherichia coli*. *Adv. Biochem Eng Biotechnol.* 85: 43-93.
- Pulliam TR, Winston S, Bentley WE (1997). Tryptophan regulated

- expression and aqueous two-phase separation of recombinant HIV-fusion peptides. *Enzyme and Microbial Technol.* 20: 46-51.
- Sambrook J, Russell DW, Sambrook J (2001). *Molecular Cloning. A Laboratory Manual*. New York, NY, USA, Cold Spring Harbor Laboratory Press. p. 999. ISBN 0879695773.
- Shimizu N, Fukuzono S, Harada Y, Fujimori K, Gotoh K, Yamazaki Y (1991). Mass production of human epidermal growth factor using fed-batch cultures of recombinant *Escherichia coli*. *Biotechnol. Bioengr.* 38: 37-42.
- Sumner JV, Somers GF (1949). In: *Laboratory of Biological Chemistry*, 2nd ed. Academic Press. Inc. New York, 38-39.
- Tanaka N, Saitoh A, Nakata A, Shinagawa H (1992). A simple method for overproduction and purification of p24 gag protein of human immunodeficiency virus type 1. *Microbiol. Immunol.* 36: 823-831.
- Yanofsky C (2004). The different roles of tryptophan transfer RNA in regulating trp operon expression in *E. coli* versus *B. subtilis*. *Trends in Genet.* 20: 367-374.
- Yanofsky C (2003). Using studies on Tryptophan Metabolism to Answer Basic Biological Questions. *The J. Biol. Chem.* 278: 10859-10878.
- Zhang GG, Rodrigues L, Rovinski B, White KA (2002). Production of HIV-1 p24 protein in transgenic tobacco plants. *Mol. Biotechnol.* 20: 131-136.