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Optimization in Transfection and Stable Production of β-galactosidase in Chinese Hamster Ovary Cells

[1]N. Saifudin, [2]N. Ibrahim and [1]N. Anuar
[1]Department of Chemical and Process Engineering, Faculty of Engineering and Built Environment,
[2]School of Biosciences and Biotechnology, Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia

Abstract: The aim for this study was to develop recombinant Chinese Hamster Ovary (CHO) cell line producing high and stable β-galactosidase by using Lipofectamine and TransFast as transfection systems. A comparison of these two gene delivery systems was made using β-galactosidase protein expression as the endpoint readout. Parameters such as lipid to DNA ratios and different amounts of Lipofectamine or DNA used were determined for optimization of transfection. In the Lipofectamine system the highest clone number was obtained from the combination of 4.5 μL Lipofectamine and 0.75 μg DNA or 2:1 charge ratio. By using TransFast, a lipid to DNA charge ratio of 2:1 is suitable for transfection. Clone TF 9 (7) that was transfected with 9 μL TransFast and 1.5 μg DNA but maintaining this 2:1 charge ratio was found to be the most productive clone in β-galactosidase production even when scaled up to 100 mL in zeocin free medium. The highest level of production for this clone and other productive clones are on the fourth day of cultivation. The highest β-galactosidase activity by subclone TF9 (7) was about 9.64 U mg^{-1} in 100 mL working volume of zeocin free medium. This study showed TransFast was more efficient than the Lipofectamine LTX for transfection and lipid mediated DNA delivery is an efficient mean for *LacZ* gene transfer into CHO cell.

Key words: CHO cells, β-galactosidase, transfection, trans fast, lipofectamine

INTRODUCTION

Mammalian cells are being used to produce numerous, high-value protein therapeutics. To understand the functions and regulations of various DNA sequences and for gene therapy, introduction of DNA into mammalian cells become a powerful tool. A large number of DNA delivery systems have been developed for mammalian cells including non biological and biological techniques. Biological technique such as viral delivery system is more efficient than non biological technique, but safety concerns regarding immunogenicity, oncogenic properties, risk of recombination with wild-type viruses and unknown long-term effects remain problematic for its potential clinical use (Mah *et al*., 2002). The non biological techniques can be divided into physical method such as electroporation (Lin *et al*., 2009), microinjection, conventional needle injection, nucleofection (Jacobsen *et al*., 2006) and chemical method such as calcium phosphate (Jordan and Wurm, 2004), DEAE-Dextran (Onishi *et al*., 2007) and cationic lipid (Henriques *et al*., 2009). When compared with biological and physical methods, the major advantages of chemical methods are their high transfection efficiency for multiple cell types, less limit on gene size, and useful for *in vitro* and *in vivo* delivery (Zhang *et al*., 2007).

Among these chemical methods, cationic lipid has become very popular method in DNA transfection. It has been shown that cationic lipid not only assists DNA into penetrating the cell, but also prevents its degradation by cellular nucleases. Nevertheless, when working at its best, cationic lipid can deliver DNA into cells more efficiently than precipitation with polycations such as calcium phosphate and at lower cost than electroporation. Though cationic lipid mediated DNA has become very popular in recent years, not all the responses are positive. The efficiency of cationic lipid was affected by a lot of factors, including types of cells line, lipid/DNA ratio, concentration of DNA, initial density of the cell culture, time of exposure of cells to the cationic lipid-DNA complex (Kaiser and Toborek, 2001) and also the purity of the DNA preparations. Moreover, cationic lipid conditions are host cell line dependent and therefore optimal cationic lipid conditions must be established for each host cell line.

One of the most important criteria for successful generation of a therapeutic protein from a recombinant cell is to obtain a cell line that maintains stability of

Corresponding Author: Nazlina Ibrahim, School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi Selangor, Malaysia
Tel: + 60389213815 Fax: + 60389252698

production. Stable transfections refer to the constant level of protein production of a population of cells for long periods of culture in which the gene of interest is stably expressed in the cell. The gene is not only introduced into the cell but also is integrated into the host genome and reproduced during cell cycles or cell division (Wurm, 2004). The process of generating a recombinant cell line involves the isolation and expansion of clonal cell line from a pool of cells transfected with the transgene and a selectable gene. The stability of recombinant cell lines is determined by monitoring cell growth and protein production for several months.

There are several studies in the literature that have reported on the instability of protein production from recombinant cell lines. The cause of instability in protein production and yield varies with molecular factor that involves vector design, plasmid integration, chromosomal environment, mRNA stability and processing, translation, secretion (Barnes and Dickson, 2006) and cell death by apoptosis (Koo *et al*., 2009).

The objective of this study is to obtain stable recombinant CHO cell clones by evaluating transfection efficiency of plasmid DNA pcDNA4/HisMax-TOPO/*lacZ* in different cationic lipid conditions of TransFast and Lipofectamine. Secondly, we then analyse the recombinant subclones for β-galactosidase production and the effect of increasing growth medium in protein expression over several passages in the presence and absence of zeocin.

MATERIALS AND METHODS

Plasmid DNA preparation: Plasmid pcDNA4/HisMax-TOPO/*lacZ* (8.35 kb) from Invitrogen (USA) is a eukaryotic expression vector containing the CMV promoter and *lacZ* gene that codes for the enzyme β-galactosidase that allows quick determination of cells expressing the gene. The plasmid was first transformed in *Escherichia coli* Top10 and prepared using the Pure Yield plasmid midiprep system (Promega, USA). The structural integrity and topology of purified DNA plasmid were analysed by 0.8% agarose gel electrophoresis. DNA concentration and purity were quantified by UV absorbance at 260 and 280 nm on a Biophotometer (Eppendorf, Germany).

Cell transfection: Two different systems for transfection optimisation used in this study were Lipofectamine LTX (Invitrogen, USA) and TransFast (Promega, USA). CHO cells were cultured in growth medium containing culture media RPMI-1640 with 5% fetal bovine serum (FBS) and 1% penicillin/streptomycin (all purchased from Gibco-BRL). The cells were seeded at 1.6×10^5 cells per well onto 12-well plates 24 h before transfection. The cells were grown to 70% confluence at the time of transfection. Then the cells were washed twice with phosphate buffered saline (PBS). For lipofectamine (Lipo) transfection, 1 mL of serum-free and antibiotics-free medium was added into each well. To optimise transfection, 10 combinations of different concentrations of plasmid DNA and different volumes of Lipofectamine were tested. The transfection mixture were overlaid and mixed gently to confluent cells. The cells were then incubated for 5 h at 37°C in an incubator with 5% CO_2. Lipofectamine was then removed after the incubation and the cell surfaces were rinsed thoroughly with PBS and 2 mL of the growth medium was added. Then the cells were further incubated for 48 h. Stable transfected cells were selected by ability to grow in zeocin (Invitrogen, USA) at 300 $\mu g\ mL^{-1}$ that was added to the growth medium for 4 weeks.

For TransFast transfection system, 10 combinations of various concentration of plasmid DNA at charge ratios (TransFast reagent to DNA) of 1:1 and 2:1 were tested. The TransFast/DNA solutions were diluted in serum free medium and mixture was incubated for 15 min at room temperature. The RPMI-1640 medium was removed from the 12-well plates containing cells, washed twice by PBS and replaced by the Trans Fast/DNA mixture. After 1 h, 1.6 mL growth medium was added and the cells were incubated for 48 h. Stable transfected cells were selected by addition of zeocin as above.

Cell cloning by limiting dilution: At the end of the weeks incubation, transfected colonies were identified morphologically and marked. The clone of transfected cells were transferred to 96 well plates and supplied with 150 μL culture medium with zeocin in each well. The cells were expanded into 12 wells plate when 100% confluence was reached. Seventy-eight clones were isolated and cultured for 2 weeks after which β-galactosidase producer cells were identified using β-galactosidase activity assay.

β-galactosidase activity assay: β-galactosidase activity assay was performed using BetaRed β-Galactosidase Assay Kit (Novagen, USA) . Cells cultured in a 12-well plate were rinsed with PBS once and 200 μL of Reportasol extraction buffer was added to each well. The plate was shaked at room temperature for 5 min. For assay protocol, 50 μL cell lysate and negative control were placed into different wells of the 96 well plates and 145 μL of Beta red reaction buffer with dithiothreitol (DTT) and chlorophenol red beta-D-galactopyranoside (CPRG) substrate was added to the wells. The plate was incubated 37°C until the

reaction changes to red. Beta Red stop buffer was added to stop the reaction and absorbance was measured at 595 nm wavelength using a microplate reader (Bio-Rad, USA).

Bradford protein assay: To determine the total amount of protein, Quickstart Bradford protein assay (Bio-Rad, USA) was performed according to the manufacturer's protocol, using bovine serum albumin (BSA, Sigma) as the standard. BSA standard (125-2000 mg mL^{-1}) and 20 μL cell lysates were incubated with working reagent at room temperature for at least 5 min before absorbance was measured at 595 nm on the Biophotometer (Eppendorf).

Western blot analysis: Cell extract was added with protein loading dye and boiled for 5 min at 100°C. A volume of 35 μL was loaded and separated by electrophoresis on 12% SDS-polyacrylamide gel. The gel was blotted onto a nitrocellulose membrane (Bio-Rad, USA). The membrane was blocked for 1 h using 5% skimmed milk in PBS with 0.05% Tween-20 and incubated overnight with anti- β-galactosidase monoclonal antibody (Promega, USA) diluted to 1:5000 in blocking buffer. After washing, the captured β-galactosidase protein was detected with horseradish peroxidase-conjugated anti-mouse IgG (H+L) (Promega, USA) diluted to 1:2500 in blocking buffer for 1 h at room temperature. The protein bands were detected using 3,3, 5,5-tetramethylbenzidine solution (Promega, USA).

RNA isolation, reverse transcription-polymerase chain reaction (RT-PCR) and amplification of β-galactosidase producer clone: Confluent CHO cell grown in T-75 flask with ~2.5×10^{7} cells were harvested. Total cellular RNA was extracted from the cells using Masterpure RNA purification kit (Epicentre Biotechnology, USA) according to the manufacturer's instructions. cDNA was amplified from 5 μg total RNA by reverse transcription (RT) using First Strand cDNA Synthesis kit (Novagen, USA) in total volume of 20 μL. The first strand cDNA synthesis was primed with a gene specific downstream primer (BGH reverse for β-galactosidase and positive control primer 3' Antisense for positive control RNA (supplied in the kit). Polymerase chain reaction (PCR) was perfomed in a total volume of 50 μL, using 5 μL cDNA from the first strand synthesis, 25 μL 2× GoTaq Hot Green Master mix (Promega, USA). For β-galactosidase gene cDNA, PCR was programmed as follows: 30 cycles of denaturation at 95°C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 2 min in the presence of 1 μM upstream primers (5'-tatggctagcatgactggt-3'). The PCR for positive control was programmed as follows: 30 cycles of denaturation at 95°C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 3 min in the presence of 25 ρmol 5' sense primer (Novagen). The PCR products were analysed by electrophoresis on 0.8%).agarose gel.

Stability of protein expression in selective and non selective medium: Two clones with highest β-galactosidase producer were cultivated for eleven passages and the stability of clones that expresses the protein when scaled up to higher volume in the presence or absence of zeocin was determined. Cells were seeded at 1.5×10^{6} cells mL^{-1} in the T25-flask with 6 mL of selective medium (RPMI 1640 + 5% FBS + 300 μg mL^{-1} zeocin) or 7.5×10^{6} cells mL^{-1} in the T75-flask with 30 mL^{-1} selective medium. Cells at the density of 2.5×10^{7} cells mL^{-1} were grown in 100 mL^{-1} of non selective medium which only contains RPMI 1640 culture medium and 5% FBS. Cells were harvested and analysed for β-galactosidase activity every day for five days to determine the growth profile.

Measurement of cell viability: The cell density was measured using a hemacytometer (Neubaeur, UK) and viable cells were detected using trypan blue (Sigma) exclusion test. Cell viability was expressed as the percentage of the number of viable cells over the total number of cells.

RESULTS

Optimisation of transfection conditions: The optimisation of the Lipo/DNA and Transfast/DNA combinations is an important prerequisite step since various media compositions contain different charged components that interfere with the complex. This can influence the transfection efficiency due to unbound Lipo and TF. Optimisation using 10 different concentration combinations of DNA and Lipofectamine LTX resulted in only three pairs of the combinations produces transfected clones implied by their ability to grow in zeocin containing medium (Table 1). The total number of clones were 10 with the highest clone number was obtained from the combination of 0.75 μg DNA and 4.5 μg Lipofectamine. Three clones were obtained from the combination of 0.75 μg DNA to 2.0 μg Lipofectamine and 2 clones were produced using 0.75 μg DNA to 1.125 μg Lipofectamine (Table 1).

Sixty eight clones survived after selection using zeocin from the TransFast transfection optimisation (Table 2). Transfection screening using TransFast system with 10 different combinations showed that the resistant clones were dependent on the plasmid integration into the CHO cell and only those cells that express the selector gene survived. The combination of charge ratio of

Table 1: Ten combinations of lipofectamine and DNA concentrations, lipo to DNA ratio and number of clones produced after the transfection

Combination	Lipofectamine LTX (μg)	DNA (μg)	Lipo to DNA ratio	Number of clones
1	0.75	0.5	1.5:1	0
2	1.00	0.5	2:1	0
3	1.75	0.5	3.5:1	0
4	2.50	0.5	5:1	0
5	3.00	0.5	6:1	0
6	1.125	0.75	1.5:1	2
7	2.00	0.75	2.7:1	3
8	2.5	0.75	3:1	0
9	3.0	0.75	4:1	0
10	4.5	0.75	6:1	5

Table 2: Ten combinations of TransFast and DNA concentration, to DNA charge ratio, number of clones produced after the transfection and clone name

Combination	Trans Fast(μL)	DNA (μg)	Charge ratio of transFast to DNA	Number of clones	Clone name
1	1.5	0.5	1 : 1	1	TF1(1)
2	3	1.0	1 : 1	0	-
3	3.75	1.25	1 : 1	0	-
4	4.5	1.5	1 : 1	1	TF4(1)
5	6	2.0	1 : 1	0	-
6	3	0.5	2 : 1	11	TF6(1)-TF6(11)
7	6	1.0	2 : 1	6	TF7(1)-TF7(6)
8	7.5	1.25	2 : 1	10	TF8(1)-TF8(10)
9	9	1.5	2 : 1	16	TF9(1)-TF9(16)
10	12	2.0	2 : 1	23	TF10(1)- TF10(23)

TransFast to DNA (2:1) using 2 μL TransFast and 2.0 μg DNA produces 23 clones. Generally, TransFast system with charge ratio of 2:1 performed better transfection capability than the combination of charge ratio 1:1. The number of surviving clones was between 6-23 clones when charge ratio 2:1 compared to only one using charge ratio of 1:1 at different DNA concentrations.

Comparison in β-galactosidase production between clones: To determine which clone would yield the highest level of recombinant protein expression, screening was done on all 78 clones. Only six clones were selected as candidate production lines. The activity and production of β-galactosidase was confirmed by the β-galactosidase assay (Fig. 1a) and Western blot analysis (Fig. 1b). Each of the transfected clones expresses β-galactosidase as a single band corresponding to 120 kDa compared to the control with no band displayed. The level of β-galactosidase detected in the cell lysate using CPRG substrate was clearly dependent upon the expression of β-galactosidase in the clones. The combination of TransFast 9 μL and 1.5 μg DNA or 2:1 charge ratio that produces clone TF9 (7) was detected to be the highest β-galactosidase producer clone (52.45 milliUnits mg^{-1}) followed by Transfast (2:1) and 1.25 μg DNA combination for subclone 1 (35.1 mU mg^{-1}). The last four clones generated roughly similar amounts of protein production ranging from 4-8 mU mg^{-1}. There was no clone that

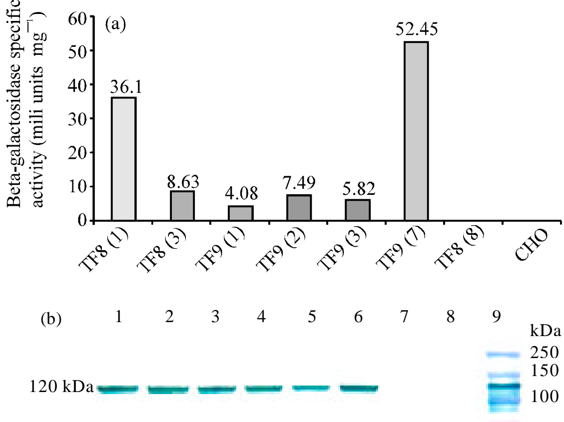
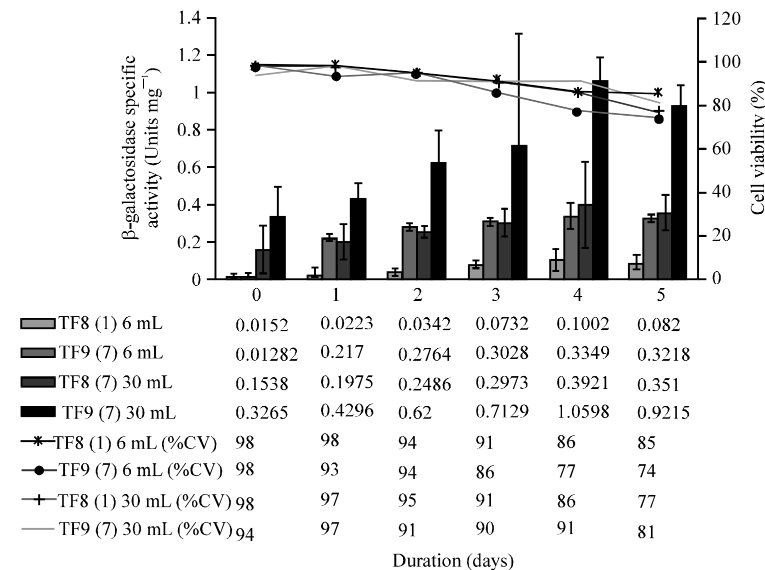


Fig. 1: Analysis of ß-galactosidase production in TransFast mediated transfected CHO cell clones from the second passage by (A) β-galactosidase specific activity assay and (B) Western blot analysis of protein (~120 kDa in size) expressed by clones resulting from different transfection combinations. Lane 1: TF8(1); 2: TF8(3); 3: TF9(1); 4: TF9(2); 5: TF9(3); 6: TF9(7); 7: TF8(8)-transfected cell but no ß-galactosidase production; 8: non-transfected CHO cells.9. Prestained protein marker

produces β-galactosidase activity from the Lipofectamine transfected clones. In this set of experiments, TransFast transfected clones were better in the ability to express β-galactosidase compared to the Lipofectamine LTX transfected CHO cell. However, only 8.8% (6 out of 68 clones) of the TransFast transfected cells were detected as the producer clones.

Establishment of stable cell lines expressing *lacZ* gene: The β-galactosidase genes transcripts were transcribed by RT-PCR to monitor expression of recombinant proteins in different cell clones. Figure 2 shows the amplicons for the β-galactosidase genes with approximate size of 3000 bp were only present in transfected cells. The expression pattern of exogenous genes transfected into mammalian cells is complex. Expression of recombinant protein depends on the number of integrated copies per genome and cell type according to Vianna *et al.* (2003). The results showed a correlation between the β-galactosidase activity and the amount of *lacZ* gene integrated into the cell's genomes, where the highest absorbance values correlate with the highest amounts of DNA mass. CHO cell clone TF8 (8) that is negative by β-galactosidase assay showed no amplification of cDNA after RT-PCR, indicating the possibility of rearrangement of the *lacZ* gene that inhibits the expression (Fig. 2, Lane 7).

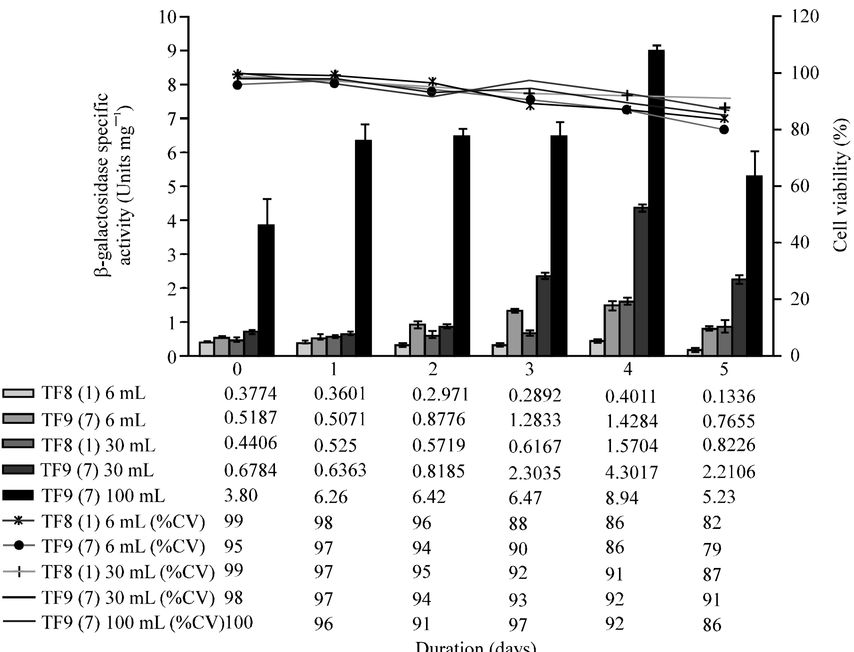
Fig. 2: RT-PCR analysis of the lacZ gene in transfected clones. Clones showed different levels of ß-galactosidase gene expression. cDNAs were amplified from total RNA extracted from transfected cells and separated by electrophoresis on 0.8% agarose gel. Lane 1: TF8(1); 2: TF8(3); 3: TF9(1); 4. TF9(2); 5: TF9(3); 6: TF9(7); 7: TF8(8); transfected cell but no ß-galactosidase production; 8: non-transfected CHO cells. 9: Positive control (G3PDH); 10: Plasmid pcDNA4/HisMax-TOPO/lacZ; 11: 1kb DNA molecular weight (Promega)



	0	1	2	3	4	5
TF8 (1) 6 mL	0.0152	0.0223	0.0342	0.0732	0.1002	0.082
TF9 (7) 6 mL	0.01282	0.217	0.2764	0.3028	0.3349	0.3218
TF8 (7) 30 mL	0.1538	0.1975	0.2486	0.2973	0.3921	0.351
TF9 (7) 30 mL	0.3265	0.4296	0.62	0.7129	1.0598	0.9215
TF8 (1) 6 mL (%CV)	98	98	94	91	86	85
TF9 (7) 6 mL (%CV)	98	93	94	86	77	74
TF8 (1) 30 mL (%CV)	98	97	95	91	86	77
TF9 (7) 30 mL (%CV)	94	97	91	90	91	81

Fig. 3: β-galactosidase production determined by the specific activity and cell viability (%) for clones TF 8(1) and TF 9(7) at passage 11 grown in selective medium (with zeocin) at different volumes. The error bars represent the standard deviations calculated from three independent experiments

Stability of protein expression in selective and non selective medium: Two of the best producing clone TF 8 (1) and TF 9 (7) were examined for stability of protein expression in the presence (with zeocin) and absence of selective pressure (without zeocin). Stability of β-galactosidase production in mammalian cell were analysed from passages 11 of each clone in 6, 30 and 100 mL (only for non selective medium) cultivation medium. Each experiment was performed at least three times. In all non-selective media, β-galactosidase expression (Fig. 3) showed higher activity than in selective media (Fig. 4). Clone TF 9 (7) gave higher activity with 8.94 Units mg^{-1} when expanded in 100 mL medium without zeocin which implies the gene of interest is stably expressed in this population of cell. Clone TF 9(7) showed consistently higher β-galactosidase expression than clone TF 8 (1). For growth in the non-selective medium, it is interesting to note that there was almost no difference in the cell viability compared to selective media (Fig. 3, 4). What is more important that the



	0	1	2	3	4	5
TF8 (1) 6 mL	0.3774	0.3601	0.2.971	0.2892	0.4011	0.1336
TF9 (7) 6 mL	0.5187	0.5071	0.8776	1.2833	1.4284	0.7655
TF8 (1) 30 mL	0.4406	0.525	0.5719	0.6167	1.5704	0.8226
TF9 (7) 30 mL	0.6784	0.6363	0.8185	2.3035	4.3017	2.2106
TF9 (7) 100 mL	3.80	6.26	6.42	6.47	8.94	5.23
TF8 (1) 6 mL (%CV)	99	98	96	88	86	82
TF9 (7) 6 mL (%CV)	95	97	94	90	86	79
TF8 (1) 30 mL (%CV)	99	97	95	92	91	87
TF9 (7) 30 mL (%CV)	98	97	94	93	92	91
TF9 (7) 100 mL (%CV)	100	96	91	97	92	86

Fig. 4: β-galactosidase production determined by the specific activity and cell viability (%) for clones TF 8(1) and TF 9(7) at passage 11 grown in nonselective medium (with no zeocin) at different volumes. The error bars represent the standard deviations calculated from three independent experiments

entire clones showed on average 85% cell viability on day five. In selective medium that contains zeocin, cell viability of more than 75% was maintained during the assay period. All clones showed maximum protein specific activity on day 4 (Fig. 3, 4).

DISCUSSION

The transfection technique is an important parameter in stable cell line generation, but up to now no comprehensive studies have been performed with different combinations amount of cationic liposome/DNA. In the last few years, cationic liposome-DNA complexes have been extensively investigated and widely used in gene therapy to deliver DNA into mammalian cells, owing to their potential advantages over viral vectors, such as their safety, versatility and low immunogenicity (Li and Huang, 2006; Lonez *et al*., 2008). This study demonstrates that lipid cationic mediated DNA delivery is an efficient mean for gene transfer into mammalian cells. Two different cationic liposomes, TransFast and Lipofectamine LTX, were utilized to compare potential difference in transfection. In this set of experiments, TransFast was more efficient than the Lipofectamine LTX.

A study by Betz and Farfan (2003) demonstrated that cells transcfected using TransFast reagent showed maximum *Renilla* luciferase reporter activity compared to other transfection reagents tested including Lipofectamine towards human neuroblastoma cell line. Lipofectamine LTX system was found to be non efficient in this study probably due to the absence of Plus reagent that may enhance cationic lipid-mediated DNA transfection by aiding in the stage in transfection where DNA is complexed to the lipids (Wong *et al*., 2007).

Rate of liposome-mediated transfection is dependent on the amount of plasmid DNA and thus on the ratio of cationic lipids to DNA. This study revealed that the transfection rate of CHO cell can be enhanced with an increase in the amount of transfected DNA up to 1.5 μg and 2:1 charge ratio of TransFast Reagent:DNA. Further increase in the amount of plasmid DNA and thus alteration of the DNA/lipid ratio, decreases the efficiency of transfection. Result obtained in the study by Betz and Farfan 2003 showed that 0.75 μg plasmid DNA and 4.6 μL TransFast Reagent at 2:1 charge ratio of TransFast Reagent to DNA was the optimum condition in 24-well tissue culture plates. Studies by Rosser *et al*. (2005); Lawless *et al*. (2004) found that a lipid to DNA ratio of 3:1 and 1:1 showed to be the most efficient for transfection and can be scaled up to liter quantities. The different lipid-mediated transfection efficiency can be affected by several factors, including cell type, culture

conditions, physico-chemical characteristics (size and zeta potential), lipid composition of the liposomes, promoter type, reporter gene type and amount of transfected plasmid DNA (Kaiser and Toborek, 2001; Zuidam and Barenholz 1998). Cationic lipid plays several roles in the process of transfection, such as condensing and protecting DNA, binding to cell surface, triggering endocytosis and releasing DNA/lipid complexes from endosome (Sun *et al*., 2004). A study by Obata *et al*. (2009) showed it is important to modify cationic lipid to enhance the gene expression of a pDNA-encapsulating liposome mediated gene delivery by varying the spacer between the cationic head group and hydrophobic moieties of the cationic lipids.

The transfection of TransFast system was found t be less efficient with only 8.8% of the survived selection were detected as the producer clones. A significant problem, as with limiting dilution methods only a few hundred clones can realistically be characterized, thus increasing the chance of missing out on high producers owing to the low number of cells screened (Browne and Al-Rubeai, 2007). Currently, the automated systems such as the ClonePix system (Genetix Ltd., United Kingdom) and the CellCelector™ (Aviso GmbH, Germany) (Hacker *et al*., 2009) is replacing the manual technique in screening clones with definitely reduce the time needed to generate recombinant cell lines and increase the probability of recovering high producing clones.

The study demonstrates that the β-galactosidase was stably maintained in expression when adapted to grow in for 11 passages. Analysis on the β-galactosidase gene transcripts agrees with stable expression of β-galactosidase protein even though when sub-cultured for several times. A study by Lattenmayer *et al*. (2007) showed correlation of mRNA levels with specific productivity of fusion protein EpoFc. However, high gene copy numbers were not always accompanied by high protein expression. Removal of zeocin as the selective pressure after the 11th passage in this study elevates expression of β-galactosidase. Removal of the selective pressure at day 28 after selection had results in the consistency of GFP expression in a previous report (Derouazi *et al*., 2006). The inconsistency might be implied by the loss of high producers due to fast adaptation to selective pressure (Reisinger *et al*., 2009). Zeocin is a member of the bleomycin/phleomycin family of antibiotics, known to bind and cleave DNA and its removal reduces DNA breakage thus increasing CHO cells activity. Trastoy *et al*. (2005) demonstrated that Zeocin, added in the culture medium to maintain the expression of the ecdysone receptor, was responsible for the formation of DNA strand breaks in the recombinant cells.

CONCLUSION

Lipid mediated DNA delivery is an efficient mean for *LacZ* gene transfer into CHO cell. This study showed TransFast was more efficient than the Lipofectamine LTX for transfection. A lipid to DNA ratio of 2:1 (12 μL TransFast and 2.0 μg DNA) was shown to be the most efficient concentration combination for transfection. However, clone TF 9 (7) that was transfected with 9 μL TransFast and 1.5 μg DNA but maintaining the 2:1 charge ratio was found to be productive in β-galactosidase production even when scaled up to 100 mL in zeocin free medium with highest level of production on the fourth day of cultivation.

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