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Production of reverse transcriptase from Moloney murine Leukemia virus in *Escherichia coli* expression system

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ABSTRACT

Reverse transcriptase (RT) is one of the most important enzymes used in molecular biology applications, enabling the conversion of RNA into complementary DNA (cDNA) that is used in reverse transcription-polymerase chain reaction (RT-PCR). The high demand of RT enzymes in biotechnological applications making the production optimization of RT is crucial for meeting the growing demand in industrial settings. Conventionally, the expression of recombinant RT is T7-induced promoter using IPTG in *Escherichia coli* expression systems, which is not cost-efficient. Here, we successfully made an alternative procedure for RT expression from Moloney murine leukemia virus (M-MLV) using autoinduction method in chemically defined medium. The optimization of carbon source composition (glucose, lactose, and glycerol) was analyzed using Response Surface Methodology (RSM). M-MLV RT was purified for further investigation on its activity. A total of 32.8 mg/L purified M-MLV RT was successfully obtained when glucose, glycerol, and lactose were present at concentration of 0.06%, 0.9%, and 0.5% respectively, making a 3.9-fold improvement in protein yield. In addition, the protein was produced in its active form by displaying 7462.50 U/mg of specific activity. This study provides the first step of small-scale procedures of M-MLV RT production that make it a cost-effective and industrially applicable strategy.

KEYWORDS

Autoinduction; chemically defined medium; *Escherichia coli*; Moloney murine Leukemia virus; reverse transcriptase

Introduction

Reverse transcriptase (RT) is a pivotal enzyme extensively employed in molecular biology and biotechnology, enabling the conversion of RNA into complementary DNA (cDNA). This enzyme firstly discovered in 1970 [1] and isolated from retrovirus [2]. Its broad applications range from cDNA synthesis for gene expression studies to reverse transcription-polymerase chain reaction (RT-PCR) for gene amplification [3], including for detecting coronavirus disease 2019 (COVID-19) with RT-PCR as gold standard [4]. RT enzyme is also the key enzyme for research in molecular tools for RNA sequencing, gene expression analysis, and cDNA cloning [3]. As the demand for RT continues to grow in industrial settings, optimizing its production and purification process becomes imperative to meet the industry's requirements efficiently [5].

Human immunodeficiency virus (HIV) [6], Moloney murine leukemia virus (M-MLV) [7], avian myeloblastosis virus (AMV) [8], and other retroviruses are potential sources of reverse transcriptase (RT). Because of its outstanding catalytic efficiency and reliable precision, the reverse transcriptase produced from Moloney murine leukemia virus (M-MLV) is the most extensively used and preferred in molecular research and laboratory applications [9]. As a result, Moloney murine leukemia virus reverse transcriptase (M-MLV RT) has considerable commercial value and shows excellent potential [10].

Our previous study [11] successfully achieved the enhancement of the recombinant enzyme of MMLV-RT in terms of both production and performance. This improvement has been demonstrated to be applicable to the RT-PCR process. Nevertheless, it's important to note that the method employed for expression remained rooted in conventional approaches, relying on Isopropyl ß-D-1-thiogalactopyranoside (IPTG) induction [12]. Conventional methods of RT production often involve induction using the T7 promoter and IPTG in *Escherichia coli* expression systems [13,14]. However, these methods have limitations such as, the high cost of IPTG as an inducer and monitoring activities is considered very inefficient [15,16].

Novel approaches have evolved to address these issues and satisfy industrial-scale needs, such as autoinduction. Instead of using T7 promoter induced by IPTG, autoinduction technique utilizes a mixture of glucose, glycerol, and lactose supplement in the media, in which lactose acts as a natural inducer [5]. When *Escherichia coli* grows, it consumes glucose first, and when glucose runs out, it is forced to use lactose, which causes T7 promoter expression while

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glycerol supports growth without inhibiting T7 protein expression [17,18].

In addition, the use of complex media for protein expression is deemed inefficient due to the significant variability in nutrient composition and quality of media, particularly in the yeast extract and peptone content [19]. This complex media comprises a heterogeneous mix of carbohydrates, amino acids, peptides, vitamins, and trace elements. The composition of yeast extract can exhibit dramatic variations owing to differences in yeast strains, yeast production processes, autolysis methods, or downstream purification steps [20]. Consequently, employing a chemically defined medium with precise control can mitigate these variations in the production of recombinant M-MLV RT in the *E. coli* expression system. This chemically defined medium application, together with the use of autoinduction method is not only will shortening production procedures, but also will make the procedure more applicable in industrial scale [5].

This study aims to present an innovative approach for the production of M-MLV RT in *Escherichia coli* expression systems, focusing on autoinduction and the use of a minimal defined medium. To improve the process even further, we used Response Surface Methodology (RSM) to optimize the carbon supply composition (glucose, lactose, and glycerol) and study the effect of the concentration of carbon source on the yield of recombinant M-MLV RT. Purification of the enzyme of interest was performed for investigation on its activity.

Material and method

Plasmid and strain

Plasmid pD451-SR_MMLV-RT harboring gene encoding reverse transcriptase from Murine Moloney Leukemia Virus (M-MLV RT) was constructed by Nuryana et al. [11]. T7 RNA polymerase (T7RNAP)-based promoter was used for the expression of recombinant protein and gene encoding kanamycin resistance was constructed on the plasmid for selection purpose. *Escherichia coli* BL21 starTM (DE3) was utilized as the host for synthesizing M-MLV RT.

Preparation of chemically defined medium

For production of recombinant M-MLV RT, a transformant colony containing plasmid pD451-SR_MMLV-RT was grown using a chemically defined medium. This medium was prepared based on Li and Sha [21] with several modifications. The chemically defined medium was prepared using the following steps: medium with a pH of 7.0 consisting of 1 x phosphate/citric acid buffer (13.3 g/L KH_2PO_4, 4 g/L $(NH_4)2HPO_4$, 1.7 g/L citric acid) and dH_2O in an erlenmeyer flask, then sterilized at 121 °C for 20 minute. After the medium temperature reached room temperature, the following compounds were added aseptically: 20 mM $MgSO_4$, 0.017 mM thiamin solution, 1x trace element solution, and various carbon sources (glucose, lactose and glycerol). 1x trace element solution taken from 100x trace element solution containing: 10 g/L Fe (III) citrate, 0.25 g/L $CoCl_2{\cdot}6H_2O$, 1.5 g/L $MnCl_2{\cdot}\ 4H_2O$, 0.15 g/L $CuCl_2{\cdot}6H_2O$, 0.3 g/L H_3BO_3, 0.25 g/L $Na_2MoO_4{\cdot}2H_2O$, 1.3 g/L $Zn(CH_3CO_2)_2{\cdot}2H_2O$, and 0.84 g/L EDTA.

Expression of recombinant reverse transcriptase using autoinduction method

Preculture medium was prepared to grow *E. coli* BL21 Star™ (DE3) harboring the plasmid pD451-SR_MMLV-RT and was incubated at 37 °C with shaking at 160 rpm. For synthesis of recombinant MML-V RT, a total of 1% (v/v) pre-culture was inoculated into 30 mL of chemically defined medium, and 200 mL culture was grown in 1 L flask with agitation at 160 rpm at 18 °C. after four days, the culture was centrifuged to separate the cell pellet from the medium at 6000 xg for 6 min at 4 °C and Tris-HCl buffer (25 mM, pH 8.0) supplemented with 1 mM PMSF was used to resuspend the cell pellet. To obtain crude extract of enzyme, sonication of the resuspended cell was performed with ultrasound probe amplitude at around 30% on ice for two times and the crude recombinant protein was separated from the cell debris by centrifugation at 18,000 xg at 4 °C for 15 min.

Experimental design

The optimal level of the carbon source in the chemically defined medium was determined to obtain the highest yield of recombinant M-MLV RT. The carbon source that was supplemented in the medium were glucose (A), lactose (B) and glycerol (C). For optimization purposes, the concentration of glucose (0.05%-0.1%), lactose (0%-0.6%), and glycerol (0%-1.2%) as shown in Table 2 were varied and the variation combination was designed in response with the yield of recombinant M-MLV RT by using Design-Expert series software, StatEase, Inc. (Minneapolis, MN, USA). Three different levels (-1, 0, +1) were used to study the effect of each factor (glucose, lactose and glycerol) on the yield of recombinant M-MLV RT including four center points, resulting in a set of 16 experiments. All the variables with central value codes as zero. Investigation on the minimum and maximum ranges of variables related to their values in coded and actual form were displayed in Table 1. The yield of recombinant M-MLV RT after four days of incubation at each 16 experiments was analyzed qualitatively with SDS-PAGE and calculated the amount of soluble M-MLV RT using imageJ software.

Validation of model

The quadratic model generated from optimal conditions based on desirability function was used for validation. Desirability range 0 d_i 1 was selected from the model to predict a set of solutions, in which individual desirability function was shown as d_i [22]. If the response reaches the goal, the value of d_i is presented as 1, whereas the value of

d_i is presented as 0 if the response is not detected in the acceptable region. Experiment using predicted model with high desirability response was conducted thrice and the value of the predicted in comparison with the value of experimental was used to evaluate the model.

Purification of recombinant reverse transcriptase

E. coli BL21 star™ (DE3) containing pD451-SR_MMLV-RT was grown in preculture medium at 37 °C with shaking at 160 rpm. Preculture (1%, v/v) was added into 200 mL of chemically defined medium and incubated four days at 18 °C with shaking at 160 rpm. The protocols for harvesting the cells and extraction of the crude recombinant protein followed the aforementioned protocols in the previous section. The crude recombinant protein was purified by applying the obtained supernatant to a HisTrap™ HP (5 mL; Cytiva, USA) column attached on AKTA Prime Plus Liquid Chromatography System (Cytiva, USA). Binding buffer containing 20 mM sodium phosphate, 500 mM NaCl, 20 mM imidazole, pH 7.4 was used to equilibrate the system. Purified fractions of recombinant M-MLV RT were obtained after elution using a linear gradient of 20–500 mM imidazole in elution buffer (20 mM sodium phosphate, 500 mM NaCl, pH 7.4) at 15 mL min^{-1} flow rate. Dialysis of the desired fractions against the 25 mM Tris-HCl buffer (pH 8.0) was performed to remove imidazole. The purity of recombinant M-MLV RT was analyzed on 10% gel by using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) following the method of Laemmli [23]. To determine the concentration of protein, bicinchoninic acid (BCA) method was employed and bovine serum albumin was used as the protein standard [24].

Table 1. Independent variables and their levels for optimization.

		Code levels		
Variable	Symbol	−1	0	+1
Glucose	A	0	0.05	0.1
Lactose	B	0	0.3	0.6
Glycerol	C	0	0.6	1.2

Table 2. Experimental design using three variables and their observed recombinant M-MLV RT production.

	A:Glucose	B:Lactose	C:Glycerol	Protein yield
Run	% (w /v)	% (w /v)	% (w /v)	g/L culture
1	0.1	0.3	1.2	39.6
2	0.05	0.3	0.6	52.2
3	0	0	0.6	0
4	0	0.6	0.6	48.4
5	0.05	0.6	1.2	44
6	0.05	0.3	0.6	49.5
7	0	0.3	1.2	46.2
8	0	0.3	0	27.7
9	0.1	0.6	0.6	48.6
10	0.1	0	0.6	0
11	0.05	0	1.2	0
12	0.05	0.3	0.6	54.2
13	0.05	0.3	0.6	52
14	0.05	0.6	0	34.6
15	0.1	0.3	0	35.9
16	0.05	0	0	0

Western blotting

To confirm the expression of recombinant M-MLV RT, western blotting was conducted in accordance with the protocol provided by HisProbe™-HRP Conjugate kit (ThermoFisher, USA) with several modifications. Electrophoresis of the purified protein was performed on 10% acrylamide gel and Mini Protean® II trans blot unit (Bio-Rad) was used to transfer the purified protein attached on the acrylamide gel to the sheet of nitrocellulose membrane. Detection of the recombinant M-MLV RT was conducted by the addition of chromogenic substance 3,3′,5,5′-tetramethylbenzidine (TMB) to the nitrocellulose membrane.

Activity assay of recombinant reverse transcriptase

The activity of recombinant M-MLV RT was quantitatively examined using EnzChek RT assay kit (Invitrogen) according to the manual protocol provided by the manufacturer. Commercial M-MLV RT (SSIV, Thermo Fisher Scientific) was serially diluted and used to produce standard curves. A total of 5 µL reaction was employed for all standards and samples. To stop the reaction, 200 mM EDTA was added into the reaction and pico green dye was used to detect DNA-RNA duplex, which was a mixture of poly(A) template, oligo-dT primer and dTTP. Fluorescence intensity was employed to examine the activity of recombinant M-MLV RT at wavelength 480 and 520 nm for excitation and emission, respectively, using a microplate reader. One unit is defined as enzyme activity which incorporate of 1 nmol of dTTP at 37 °C for 10 minutes using oligo(dT) as a primer and poly(A) as template.

Results

Expression of recombinant reverse transcriptase using autoinduction method

Autoinduction method was selected to express recombinant M-MLV RT. Instead of complex medium, *E. coli* BL21 star™ (DE3) containing pD451-SR_MMLV-RT was cultured for four days at 18 °C in chemically defined medium supplemented with 0.3% lactose as inducer for autoinduction. Recombinant M-MLV RT constructed by Nuryana et al [11] was used in this study and it was reported to possess a molecular mass of about 58 kDa. For expression assessment, *E. coli* BL21 star™ (DE3) without insertion of pD451-SR_MMLV-RT was employed as negative control and the control was cultured in the same condition with a recombinant cell. Supernatant of the sample and negative control after sonication was applied for SDS-PAGE analysis. Analysis on SDS-PAGE showed that a thick band at around 58 kDa was found as soluble form, whereas no overexpressed protein at similar size was detected in negative control, indicating that recombinant M-MLV RT was successfully overexpressed by following autoinduction method in chemically defined medium (Figure 1).

Optimization of carbon source in defined medium using RSM

Statistical analysis

To obtain higher yield of recombinant M-MLV RT, optimum concentration of glucose, lactose and glycerol supplemented in the chemically defined medium was determined using RSM (Table 1). The yield of recombinant M-MLV RT at each variation of experimental design was shown in Table 2. The F-test for analysis of variance (ANOVA) was employed to evaluate the statistical significance of the model (Table 3). The p-value of the regression model was <0.0001 suggesting that the model used in this study was statistically significant with a 99.99% confidence interval. The F-value of the model was 59.23 indicating the significance of the model. The model inability to depict data in regression that are positioned in the excluded region is measured as a lack of fit value [25]. An insignificant value of a lack of fit (p = 0.0941) was observed, suggesting that the prediction of recombinant M-MLV RT yield based on the model equation could be performed at any variable interactions. The coefficients R^2, adjusted R^2, and predicted R^2 was analyzed to test the goodness-of-fit of the model (Table 4). The result showed that R^2 was 0.9889 implying the correlation between the predicted and experimental values was high (Figure 2). The difference between the adjusted R^2 (0.9722) and predicted R^2 (0.8457) was less than 0.2 demonstrating a reasonable agreement between those two coefficients. The obtained ration was 20.1016, which was greater than 4, indicating an

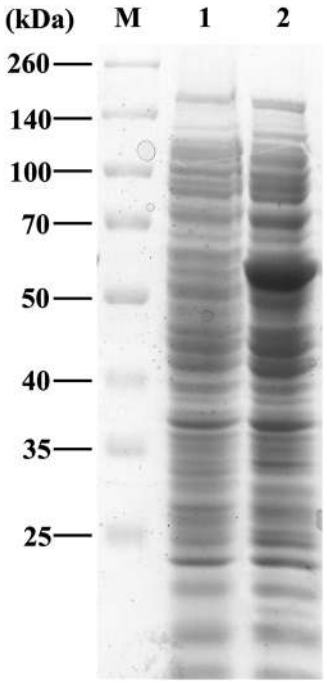

Figure 1. Analysis of recombinant M-MLV RT expression employing autoinduction method in *E. coli* system using SDS-PAGE. The M-MLV RT band was shown as a triangle symbol. Lane M: marker, lane 1: soluble fraction of E. coli BL21 starTM (DE3) as a negative control (no induction), lane 2: soluble fraction of E. coli BL21 star™ (DE3) harboring the plasmid pD451-SR_MMLV-RT (induction).

Table 4. Statistical analysis of the goodness-of-fit of the model.

Std. Dev.	0.0035	R^2	0.9889
Mean	0.0333	Adjusted R^2	0.9722
C.V. %	10.56	Predicted R^2	0.8457
		Adeq Precision	20.1016

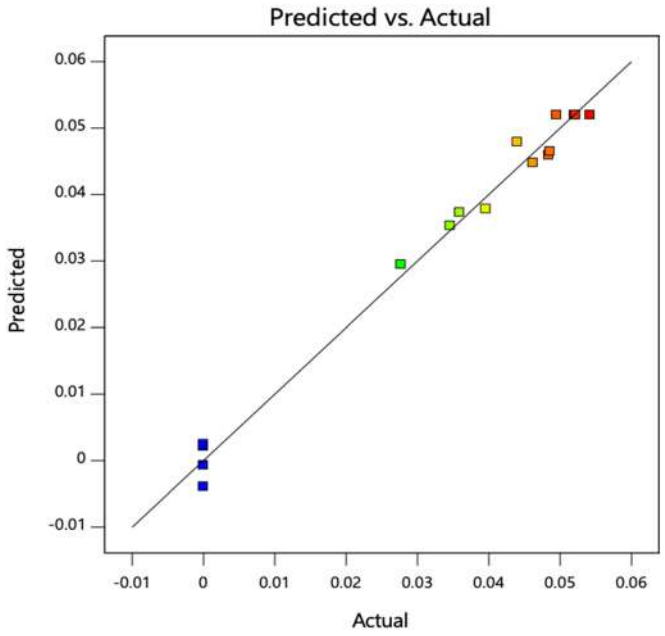

Figure 2. Predicted response versus actual values. The actual value was obtained by synthesis the target protein using following condition: a 1% (v/v) pre-culture was added to 30 mL of a chemically defined medium and a 200 mL culture was grown in a 1 L flask that was stirred at 160 rpm and kept at 18 °C for four days.

Table 3. Regression analysis result of experimental design.

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	0.0066	9	0.0007	59.23	<0.0001
A-Glucose	4.050×10^{-7}	1	4.050×10^{-7}	0.0328	0.8623
B-Lactose	0.0039	1	0.0039	311.79	<0.0001
C-Glycerol	0.0001	1	0.0001	10.10	0.0191
AB	1.000×10^{-8}	1	1.000×10^{-8}	0.0008	0.9782
AC	0.0001	1	0.0001	4.43	0.0800
BC	0.0000	1	0.0000	1.79	0.2298
A^2	0.0001	1	0.0001	8.13	0.0291
B^2	0.0021	1	0.0021	166.92	<0.0001
C^2	0.0004	1	0.0004	29.90	0.0016
Residual	0.0001	6	0.0000		
Lack of Fit	0.0001	3	0.0000	5.67	0.0941
Pure Error	0.0000	3	3.709×10^{-6}		
Cor Total	0.0067	15			

adequate signal. A second-order polynomial equation (Eq. 1) was obtained from the regression model to predict the yield of recombinant M-MLV RT and it was written as follows:

$$\begin{aligned}&\text{Yield of protein}\left(\text{g / L culture}\right), Y\\&= -0.012788 + 0.278000\text{x A} + 0.216583\text{ x B} + 0.040875\text{ x C}\\&+0.003333\text{ AB} - 0.123333\text{ x AC} + 0.013056\text{ x BC}\\&-2.00500\text{x A}^2 - 0.252361\text{ x B}^2 - 0.026701\text{ x C}^2,\end{aligned} \quad (1)$$

where Y is the yield of recombinant M-MLV RT (g/L culture), A is glucose concentration (%), B is lactose concentration (%) and C is glycerol concentration (%).

Interaction study of carbon sources in the chemically defined medium

The 3-D surface plot of the yield of recombinant M-MLV RT in correlation with concentration of lactose and glycerol at constant glucose concentration was depicted in Figure 3A. As illustrated in the figure, the yield of recombinant protein showed a positive impact with increased concentration of lactose from 0 to 0.5%. However the further increased on the lactose concentration led to a decrease in the protein yield. In contrast, glycerol had no effect on the yield of the recombinant protein. Figure 3B demonstrated the impact of glucose and glycerol concentration in the recombinant M-MLV RT production, by keeping the lactose concentration constant. It clearly indicated that an enhancement of glycerol and glucose by up to 0.9% and 0.06%, respectively, caused the increase of recombinant protein yield. Nevertheless, higher concentration of glycerol and glucose had a negative effect on the protein yield. The influence of glucose and lactose concentration on the protein production at the same concentration of glycerol was investigated (Figure 3C). The improvement on the yield of recombinant M-MLV RT was observed with the increase of lactose concentration by up to 0.5% and the higher

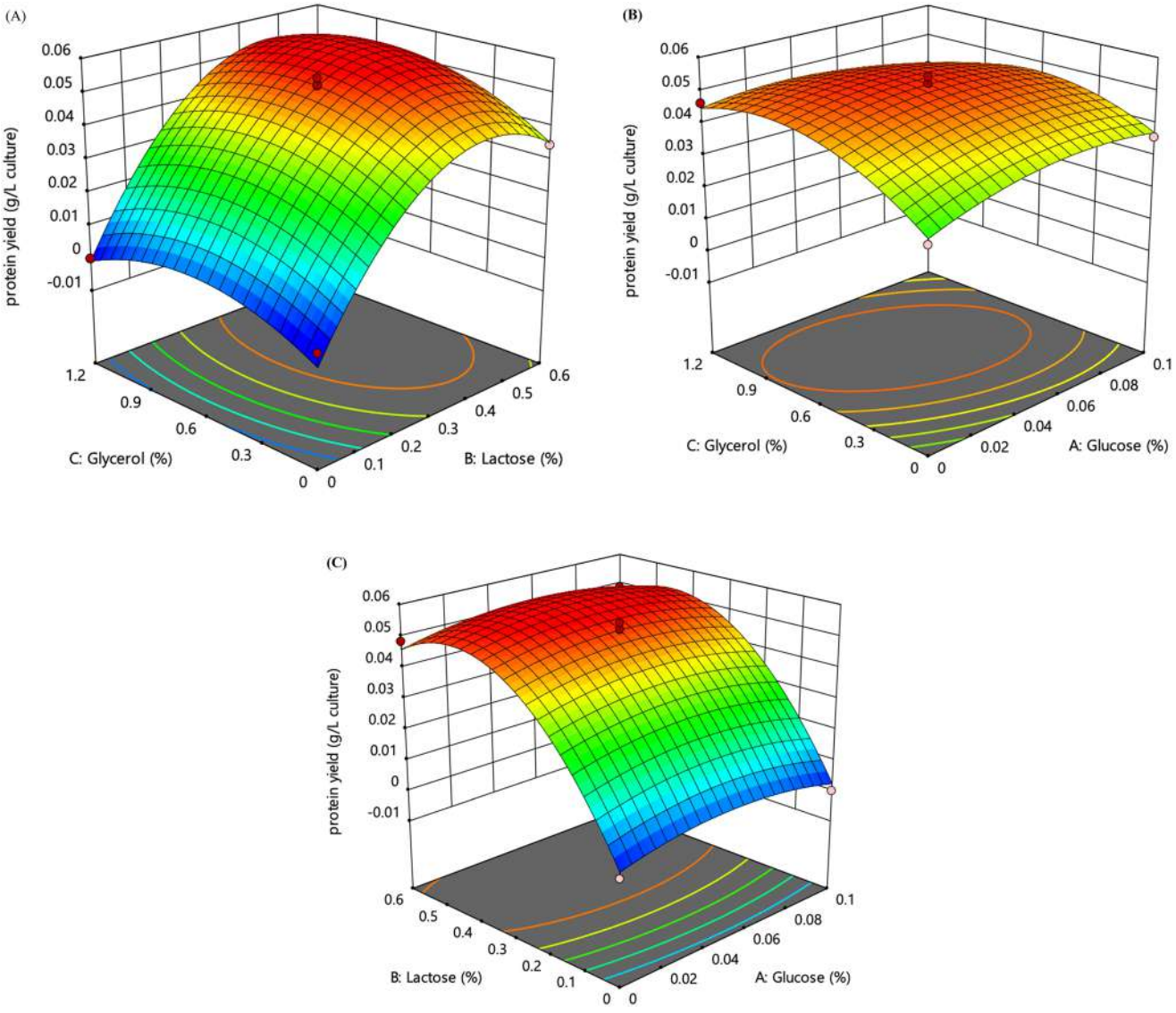

Figure 3. 3D response surface for recombinant M-MLV RT production using autoinduction method in chemically defined medium. (A) the effect of glycerol and lactose, (B) glycerol and glucose, (C) lactose and glucose, on the yield of M-MLV RT. The target protein was obtained using following condition: a 1% (v/v) pre-culture was added to 30 mL of a chemically defined medium and a 200 mL culture was grown in a 1 L flask that was stirred at 160 rpm and kept at 18 °C for four days.

concentration than that decrease the protein yield. On the contrary, glucose had no influence on the yield of protein recombinant.

Validation of the model

Based on the prediction by model, recombinant M-MLV RT reached its highest yielded 0.0379 g/L when the concentration of glucose, lactose and glycerol in the chemically defined medium were 0.038%, 0.222% and 0.223%, respectively (Table 5). The selected parameters was offered by the system and this variation of parameters had a desirability value 1.0. For confirmation, experimental production of recombinant M-MLV RT was performed by using the chemically defined medium supplemented with the same concentration of glucose, lactose and glycerol. Experimental investigation showed that at the given concentration of carbon source, the mean of recombinant M-MLV RT yield was 0.0407 g/L culture. Determination on the prediction result suitability compared with experimental result was performed using T test on GraphPad Prism. The statistical analysis showed that the p-value was 0.0646. The p-value more than 0.05 ($P > 5\%$) suggests that the value of experimental and prediction were not significantly different. This results confirms the suitability of the model as well as the solution of the independent variable.

Table 5. Validation of the response surface model against most desirable experimental conditions.

	Variables			Response
	A:Glucose (%)	B:Lactose (%)	C:Glycerol (%)	Protein yield (g/L culture)
1	0.038	0.222	0.223	0.0393
2	0.038	0.222	0.223	0.0418
3	0.038	0.222	0.223	0.0410
Average				0.0407
Results predicted with maximum desirability				0.0379
p-value				0.0646

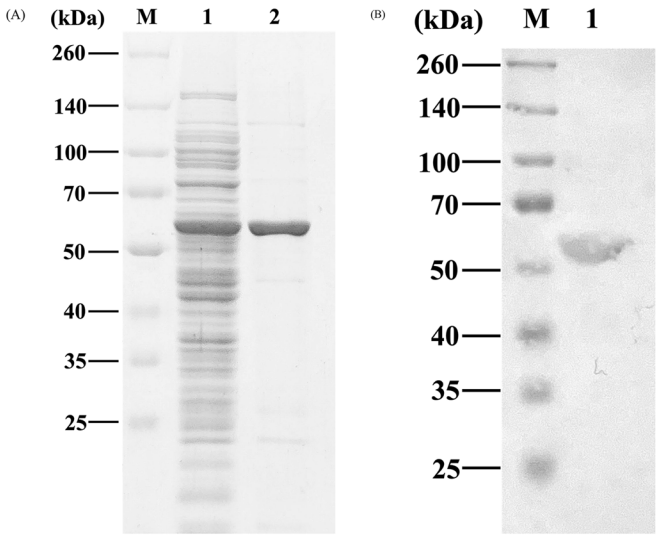

Figure 4. (A) SDS-PAGE analysis of purified M-MLV RT. Lane M: marker; Lane 1: crude protein, Lane 2: purified M-MLV RT (B) Western Blot of M-MLV RT. Lane M: marker; Lane 1: purified fraction of M-MLV RT.

Purification and activity of recombinant reverse transcriptase

Before determining the activity of recombinant M-MLV RT, purification was employed to obtain a pure enzyme. Recombinant M-MLV RT was produced in the system expression of *E. coli* BL21 star™ (DE3) in a scale-up medium, 200 mL of LB-kanamycin and the crude enzyme was purified by HisTrap™ HP. The purified fraction was analyzed using SDS-PAGE, and it showed that a high purity of recombinant M-MLV RT was successfully obtained (Figure 4A). The molecular weight of recombinant M-MLV RT was approximately 58 kDa based on SDS-PAGE analysis, which was in agreement with the predicted molecular weight from the sequence of amino acids, including His-Tag and enterokinase site at the N-terminal of the recombinant protein [11]. Pure fraction of recombinant M-MLV RT was subsequently used for confirming the expression of the enzyme by employing western blot (Figure 4B). The result of western blot analysis showed that recombinant M-MLV RT was successfully expressed in the *E. coli* expression system. As shown in Table 6, one step purification resulted in 7.48% yield of the purified recombinant M-MLV RT with over 4-fold purification. A total of 32.8 mg of purified recombinant M-MLV RT was successfully obtained from 1 L culture. Afterwards, by using both of the crude and purified enzymes, the activity of recombinant M-MLV RT was quantitatively determined. Table 6 displayed that total activity of the enzyme was 472790 and 110160 U/L culture for crude and purified enzymes, respectively. In addition, a specific activity of about 7462.50 U/mg was obtained after purification of recombinant M-MLV RT.

Discussion

Isopropyl β- d-1-thiogalactopyranoside (IPTG) is a synthetic analogue of lactose that was commonly used as an inducer for synthesizing heterologous protein in the expression system of *E. coli* with lac-derived promoter, namely induction method [26,27]. Moreover, IPTG was reported as a potentially toxic inducer as it was likely to negatively affect the cell growth [28–30]. These traits limit the application of IPTG for production of recombinant protein in the scale-up level. Development of autoinduction method for protein expression in *E. coli* system by [5] addressed the limitation of induction method due to elimination of cell growth tracking process and the employment of inexpensive lactose as a natural inducer in place of high-cost IPTG, make it a better suited method for pilot-scale fermentation. Since then, recombinant protein for various purpose has been reported to be synthesized using this method and, in several cases, the high-level expression of proteins were successfully obtained [31–34].

Protein expression in the autoinduction technique relies on the regulatory elements of lac operon, such as glucose, glycerol and lactose under two phases cellular growth allowing the transition from the uninduced state to the induced state fully controlled by the expression host [5, 17, 35]. In the uninduced state, glucose is firstly consumed until depleted before shifting to the consumption of lactose and glycerol in the

Table 6. Purification of recombinant M-MLV RT.

Process	Total activity (U)	Total protein (mg/100 mL culture)	Specific Activity (U/mg)	Purification (fold)	Yield (%)
Crude Enzyme	47279	79.47	1853.18	1	100
HisTrap™ HP	11016	3.28	7462.50	4	7.48

induced state. Depletion of glucose causes the lifting of catabolite repression and subsequently leads to the utilization of lactose as inducer, whereas the growth of the cell is backed up by glycerol [17,18]. Therefore, in the autoinduction method, a mixture of glucose, glycerol and lactose is commonly supplemented in either a complex or chemically defined medium [36]. In this study, chemically defined medium was used to produce the recombinant M-MLV RT employing *E. coli* BL21 star™ (DE3) as expression host to ensure the consistency of protein yield. The result after optimization showed that the highest yield of protein was detected when the concentration of glucose, glycerol, and lactose supplemented in the medium were 0.06%, 0.9%, and 0.5%, respectively.

Previously, Studier [5] reported that 0.05% of glucose was sufficient to be supplemented in auto-inducing media. The same case was reported for the production of [U15N]- and [U13C, U15N]-labeled proteins [37] and pullulanase [38], and thereby, similar to the result of our investigation (0.06%). This amount of glucose was able to generate a moderate density of culture and at the same time, prevented an increase of target protein basal level at the initial phase of culture growth. When shifting from non-inducing phase to the inducing phase due to the depletion of glucose, the growth of culture was supported by glycerol. Compared with glucose, glycerol did not strictly suppress the utilization of other carbon sources for the inducing activity, allowing the utilization of lactose as an inducer for the expression of target protein [5]. However, similar to glucose, high concentration of glycerol led to the generation of excess acid that stopped the growth of culture. In this study, a concentration of 0.9% glycerol was sufficient to prevent the generation of excess acid as well as support the growth of the culture. Although the usual autoinduction media contained 0.5% glycerol and 0.2% lactose [5], a higher concentration of glycerol and lactose by up to 0.9% and 0.5%, respectively, yield to the optimum amount of recombinant M-MLV RT in this present study. Similar result was shown for the production of enhanced green fluorescent protein (eGFP) using autoinduction media, 0.9% glycerol and 0.45% lactose supplemented in the medium was needed to obtain optimum protein yield [17].

In our previous study, A total of 8.4 mg/L purified recombinant M-MLV RT was obtained by using an induction method [11]. However, our approach in this study further increased the yield of the protein target. The purified recombinant M-MLV RT was 32.8 mg/L culture, which was 3.9-fold higher compared with protein target obtained from previous approach. In addition, our study demonstrated positive results compared with similar studies. Co-expression of M-MLV RT with chaperon was reported greatly improving the yield of the purified target protein by up to 21 mg/L culture [38]. From this point of view, both facilitated the significant increase of the recombinant M-MLV RT via different approaches. Other production of recombinant reverse transcriptase using induction method were also reported by Lu et al (0.075 mg/L) [39] and Silprasit (3.8 mg/L) [40] with lower protein yield. It indicates that the strategy utilized in this study is not only cost-efficient but also resulted in a high-level expression of recombinant protein.

Conclusion

A high-level expression of recombinant M-MLV RT was successfully obtained by employing the autoinduction method using optimum concentration of carbon sources supplemented in chemically defined medium. Highest yield of the purified M-MLV RT of about 32.8 mg/L culture was detected when a concentration of glucose, glycerol and lactose contained in chemically defined medium were 0.06%, 0.9%, and 0.5%, respectively. It suggested an improvement of the target protein yield by up to 3.9-fold. The result demonstrated here indicated a potential approach in producing recombinant protein suitable for industrial application.

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Ethical approval

There are no studies conducted by any of the authors in this article that used humans or animals as subjects.

Authors' contributions

YN performed data analysis and drafted the manuscript. FAL designed the research conceptualization, provided the resources, performed data analysis and validation and drafted the manuscript. H and IN performed data analysis and manuscript editing. FNK conducted the experiments and collected the data, while SS and I performed data validation and manuscript editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

All data generated or analyzed during this study are included in this article.

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