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THE EFFECT OF CONCENTRATION AND TIME VARIATION OF THE LACTOSE AND SKIM MILK AS INDUCER ON THE EXPRESSION OF RECOMBINANT PROTEIN BOVINE LACTOFERRIN CARBOXY-TERMINUS (BLF CAR)

Pengaruh Variasi Konsentrasi dan Waktu Induksi Laktosa dan Susu Skim terhadap Protein Rekombinan *Bovine Lactoferrin Carboxy-Terminus* (bLf CAR)

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Abstract

Recombinant bovine lactoferrin (bLf) production has broad potential applications in animal health, particularly as a feed additive and immunomodulatory agent, thereby requiring an efficient and sustainable production system. Lac operon–based expression systems commonly employ isopropyl β-D-1-thiogalactopyranoside (IPTG) as an inducer; however, its high cost and potential to induce metabolic stress limit its applicability for large-scale production. This study aimed to evaluate lactose and skim milk as alternative inducers for recombinant bLf expression. A synthetic bLf CAR gene was cloned into the pGEX-2TK plasmid to generate a GST-bLf CAR fusion protein and expressed under a lac operon–based system. Induction was performed using lactose and skim milk at concentrations of 1%, 2.5%, and 5%, with induction times of 5, 24, and 48 hours, while 0.1 mM IPTG served as the reference inducer. The experimental design followed a randomized block design. Protein expression was analyzed using dot blot, and signal intensity was quantified using ImageJ software. Statistical analysis was conducted using two-way ANOVA followed by Tukey ’s post hoc test. The results demonstrated that inducer type, concentration, and induction time significantly affected the expression intensity of recombinant GST-bLf CAR protein ($p < 0.01$). Skim milk at a concentration of 2.5% with a 5 hour induction period produced the highest expression level and differed significantly from other treatments. These findings indicate that skim milk is a promising, economical, and practical alternative inducer for recombinant bLf production.

Keywords: bLf CAR, IPTG, lactose, pGEX-2TK, skim milk

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Abstrak

Produksi protein rekombinan *Bovine lactoferrin* (bLf) memiliki potensi luas dalam bidang kesehatan hewan sebagai *feed additive* dan sebagai imunomodulator. sehingga diperlukan sistem produksi yang efisien dan berkelanjutan. Sistem ekspresi berbasis *lac operon* umumnya menggunakan *isopropyl β-D-1-thiogalactopyranoside* (IPTG) sebagai induktor, namun biaya tinggi dan potensi stres metabolik membatasi penerapannya pada skala besar. Penelitian ini bertujuan mengevaluasi laktosa dan susu skim sebagai alternatif induktor dalam ekspresi protein rekombinan bLf. Gen bLf CAR hasil sintesis disisipkan ke dalam plasmid pGEX-2TK untuk menghasilkan protein fusi GST-bLf CAR dan diekspresikan dalam sistem *lac operon*. Induksi dilakukan menggunakan laktosa dan susu skim dengan variasi konsentrasi 1%, 2,5%, dan 5%, serta waktu induksi 5, 24, dan 48 hour, dengan IPTG 0,1 mM sebagai pembanding. Rancangan penelitian adalah Rancangan Acak Kelompok. Ekspresi protein dianalisis menggunakan metode dot blot. Intensitas sinyal dikuantifikasi menggunakan perangkat lunak *ImageJ*. Data dianalisis menggunakan *Two-Way ANOVA* dan uji lanjut *Tukey* . Hasil penelitian menunjukkan bahwa jenis induktor, konsentrasi, dan waktu induksi berpengaruh signifikan terhadap intensitas ekspresi protein rekombinan GST-bLf CAR ($p < 0,01$). Perlakuan susu skim 2,5% pada waktu induksi 5 hour menghasilkan intensitas ekspresi tertinggi dan berbeda nyata dibandingkan perlakuan lainnya. Dengan demikian, susu skim berpotensi digunakan sebagai induktor alternatif yang lebih ekonomis dan aplikatif dalam produksi protein rekombinan bLf.

Kata kunci: bLf CAR, IPTG , laktosa, pGEX-2TK, susu skim

INTRODUCTION

Bovine lactoferrin (bLf) is a bioactive protein with broad potential in the fields of animal health, feed additives, and modern biotechnology. This protein acts as an immunomodulator, antimicrobial, antiviral, and anti-inflammatory agent through its effective iron-binding mechanism (Cui *et al.*, 2024). These high biological values are driving increased demand for bLf in the global market, which reached 297.3 million USD in 2024 and is projected to rise to 411.8 million USD by 2028 (Grand View Research, 2023). This situation indicates that the availability of bLf in large and sustainable quantities is a strategic necessity, particularly through the development of efficient recombinant protein production systems.

Recombinant bLf protein production is typically carried out using a lac-operon-based expression system with Escherichia coli as the host (Sun *et al.*, 2024). In line with this, the Faculty of Veterinary Medicine at Udayana University developed the pGEX-2TK-bLf plasmid, which engineers the bLf protein into a fusion form with Glutathione S-transferase (GST). This system is designed in two fragments, namely the amino-terminus and carboxy-terminus (CAR), thereby facilitating the expression and purification of the recombinant protein. The lac operon expression system requires Isopropyl-β-D-thiogalactopyranoside (IPTG) as a standard inducer due to its stability and the fact that it is not metabolized by the cells (Lucero *et al.*, 2021). However, the large-scale use of IPTG faces several limitations, including relatively high costs, potential environmental impacts, and the risk of metabolic stress on host cells (Gomes *et al.*, 2020; Zhou *et al.*, 2024). Given these limitations of IPTG, attention has shifted toward the use of alternative inducers that are more natural, economical, and environmentally friendly (Greicius *et al.*, 2023). Lactose and skim milk have emerged as promising alternative inducer candidates because they can physiologically activate the lac operon system (Khani & Bagheri, 2020; Wurm *et al.*, 2016). In addition to containing lactose as an inducer molecule, skim milk also provides supporting nutritional components that have the potential to maintain cellular metabolic stability during the induction process (Shah *et al.*, 2022).

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Although some studies have reported the potential of lactose and skim milk as alternative inducers, information regarding optimal concentrations and induction times remains limited, and no standard protocols are currently available, particularly for the expression of the recombinant GST-bLf CAR protein. Therefore, this study was conducted to investigate the effects of varying concentrations and induction times of lactose and skim milk on the recombinant GST-bLf CAR protein. In addition, this study also compared the expression quality of the recombinant GST-bLf CAR protein induced with 0.1 mM IPTG, lactose, and skim milk using dot blot analysis as a simple yet effective immunodetection method.

RESEARCH METHODS

Ethical suitability of laboratory animals

This study did not require ethical approval regarding animal welfare because all stages of the research were conducted in vitro without involving the use, handling, or intervention of live animals.

Research Object

The subject of this study is the recombinant GST-bLf CAR protein expressed in *E. coli* BL21 bacteria. The vector used is the pGEX-2TK plasmid, which carries the bLf CAR gene.

Research Design

This study is an experimental study designed using a Randomized Block Design (RBD), with induction times (5, 24, and 48 hours) as the blocks. Grouping based on induction time was performed to control for this variation, so that the effects of inducer type and concentration on recombinant protein expression could be observed more accurately. This study consists of four treatment groups: a negative control comprising *E. coli* BL21 cultures without a plasmid in TBG medium; a positive control comprising *E. coli* BL21 cultures carrying the pGEX-2TK-bLf CAR plasmid and induced with 0.1 mM IPTG in TBG medium; lactose induction treatment with concentration variations of 1%, 2.5%, and 5% in TB medium, and skim milk induction treatment with concentration variations of 1%, 2.5%, and 5% in TB medium. The total number of research samples was 72, consisting of 9 negative control samples, 9 IPTG-positive control samples, 27 lactose treatment samples, and 27 skim milk treatment samples.

Research Variables

The independent variables in this study included the type of inducer—namely IPTG, lactose, and skim milk—the inducer concentration—1%, 2.5%, and 5% for lactose and skim milk, and 0.1 mM for IPTG—and the induction time—5 hours, 24 hours, and 48 hours. The dependent variable in this study is the expression level of the recombinant GST-bLf CAR protein, determined based on the signal intensity from dot blot analysis. The control variables include incubation temperature, culture medium composition, host bacterial strain type, and medium pH.

Preparation of TB and TBG Media

Terrific Broth (TB) and Terrific Broth Glycerol (TBG) media were prepared by dissolving 23.5 g of TB media powder (Invitrogen) in 500 mL of sterile distilled water. To prepare TBG, 2 mL of glycerol was added after the medium was homogenized. The medium was then heated until completely dissolved and sterilized using an autoclave at 121°C for 15 minutes before being used in the bacterial culture process.

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Preparation of Lactose and Skim Milk Inducer Solutions

Lactose and skim milk inducer solutions were prepared by dissolving 125 g of lactose powder or skim milk powder, respectively, in 500 mL of sterile distilled water to obtain a 25% concentration. The solution is homogenized until completely dissolved, then sterilized using an autoclave at 121°C for 15 minutes. Solutions with concentrations of 1%, 2.5%, and 5% are prepared by diluting the lactose or skim milk solution.

bLf CAR Culture

2 µL of *E. coli* BL21 culture carrying the pGEX-2TK-bLf CAR plasmid was added to 6 mL of TBG medium containing 60 µL of ampicillin. The culture was incubated at 37 °C at 120 rpm for 48 hours. A negative control culture used *E. coli* BL21 without the plasmid and without the addition of antibiotics.

Induction Using IPTG, Lactose, and Skim Milk

The expanded bacterial cultures were centrifuged to obtain a cell pellet, which was then resuspended in TB medium according to the inducer treatment. Induction was performed using 0.1 mM IPTG as a positive control, as well as lactose and skim milk at concentrations of 1%, 2.5%, and 5%. The volume of the inducer was calculated using a dilution formula to achieve the desired final concentration. Samples were incubated and collected at 5 hours, 24 hours, and 48 hours for further analysis.

Confirmation of Protein Expression Intensity via Dot Blot

The expression intensity of the recombinant GST-bLf CAR protein was confirmed using the dot blot method. Samples were mixed with buffer and heated to boiling for 8 minutes according to the protocol of the Biomedical Laboratory, Faculty of Veterinary Medicine, Udayana University. The samples were then spotted onto a nitrocellulose membrane. The membrane was blocked using a 5% skim milk solution and washed with PBST, followed by incubation with anti-GST primary monoclonal antibody and anti-mouse IgG secondary antibody. Protein signal visualization was performed using alkaline phosphatase substrate until a color appeared indicating the target protein.

Quantification of Dot Blot Signal Intensity Using ImageJ

Dot blot signal intensity was quantitatively analyzed using ImageJ software. Dot blot images were converted to grayscale and inverted to enhance contrast. Each signal spot was analyzed using a uniform measurement area, and the Integrated Density value was recorded as the intensity level of recombinant GST-bLf CAR protein expression.

Data Analysis

Dot blot signal intensity data from all treatments were tabulated and statistically analyzed. The Shapiro–Wilk normality test was performed to evaluate data distribution, and the main analysis used a Two-Way ANOVA with inducer concentration and induction time as factors to assess the main effects and their interactions on the expression intensity levels of the recombinant GST-bLf CAR protein. If significant differences were found, the analysis was continued with Tukey's Honestly Significant Difference (Tukey HSD) post-hoc test to compare between treatments. The significance level was set at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Results

E. coli BL21 cultures carrying the pGEX-2TK-bLf CAR plasmid grew well in the culture

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medium prior to the induction process. All cultures exhibited relatively uniform turbidity levels, indicating that bacterial growth proceeded normally and homogeneously across all treatments, as shown in Figure 1. This condition serves as a crucial foundation to ensure that the differences in protein expression observed in subsequent stages are not influenced by variations in initial culture growth. After the induction process using IPTG, lactose, and skim milk, the expression of the recombinant GST-bLf CAR protein was analyzed using the dot blot method. The dot blot results are shown in Figure 2, which reveals signals in the form of brownish-black circles with varying intensities across different inducers, concentrations, and induction times.

The results of dot blot intensity measurements, quantified using ImageJ software, for the expression of the recombinant GST-bLf CAR protein in culture over 48 hours, with observation times of 5 hours, 24 hours, and 48 hours after induction with 0.1 mM IPTG, 1%, 2.5%, and 5% lactose, as well as 1%, 2.5%, and 5% skim milk, are shown in Table 1. The induction treatment using 2.5% skim milk showed the highest average GST-bLf CAR protein expression intensity compared to the other induction treatments. The bar chart in Figure 3 provides a clearer illustration of the differences in the average intensity of GST-bLf CAR protein expression among the treatments.

A normality test was performed using the Shapiro–Wilk test; Table 2 shows that not all treatment groups were normally distributed ($p < 0.05$). The results of the Two-Way ANOVA analysis shown in Table 3 indicate that the factors of inducer concentration and induction time have a highly significant effect on the expression intensity of the recombinant GST-bLf CAR protein ($p < 0.01$). Furthermore, the inducer, concentration, and induction time interact in a highly significant manner ($p < 0.01$). The results of the Tukey HSD post-hoc test in Table 4 indicate that the interaction between induction time and inducer concentration resulted in a significant difference in the expression intensity of the recombinant GST-bLf CAR protein. Differences in dot blot intensity among inducers, concentrations, and induction times varied. Furthermore, the 2.5% skim milk treatment produced the highest expression intensity of the recombinant GST-bLf CAR protein at 5 hours and 48 hours.

Discussion

The production of the recombinant bLf protein via bacterial expression systems continues to be developed to achieve efficient, economical, and sustainable methods. One of the main challenges in *E. coli*-based expression systems is the selection of an inducer capable of optimally activating the lac operon system without imposing an excessive metabolic burden on the host cells (Lucero *et al.*, 2021). Therefore, this study evaluates the effectiveness of lactose and skim milk as alternative inducers to IPTG in expressing the recombinant GST-bLf CAR protein in *E. coli* BL21.

In this study, the expression levels of the recombinant GST-bLf CAR protein were analyzed using the dot blot method, with signal intensity quantified using ImageJ software. This method was chosen because it has good sensitivity in detecting differences in signal intensity between treatments and is widely used as a quantitative approach in protein expression analysis (Puzari *et al.*, 2025; Zhuang *et al.*, 2025). The quantification process was performed in a standardized manner through image inversion, background correction, and measurement of the Integrated Density value with a consistent selection size for each dot, so that the results obtained could be objectively compared across treatments.

Before statistical analysis was performed, the data on the expression levels of the recombinant GST-bLf CAR protein were first tested for normality using the Shapiro–Wilk test. The test results indicated that most of the data were not normally distributed; however, the analysis proceeded using a Two-Way ANOVA because the study design was balanced, with three

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replicates for each treatment combination, as performed by the following researchers (Knief & Forstmeier, 2021). In a balanced study design, ANOVA is known to be quite robust to violations of the normality assumption, especially when the analysis focuses on comparing means and the treatment effects are relatively large (Blanca *et al.*, 2017). The results of the Two-Way ANOVA analysis showed that inducer type, inducer concentration, and induction time had a highly significant effect on the expression intensity of the recombinant GST-bLf CAR protein ($p < 0.01$), and indicated a highly significant interaction between inducer concentration and induction time.

These results indicate that the success of protein expression is determined by a combination of induction conditions, rather than by a single factor. Inducer concentration controls the level of promoter activation and the number of gene transcripts produced (Kim *et al.*, 2004). Too low a concentration results in suboptimal induction, whereas too high a concentration can increase the metabolic burden and trigger the formation of inclusion bodies, which reduces protein expression efficiency (Friedman *et al.*, 2024).

The induction time factor exhibits a non-linear pattern of change. A decrease in expression at 24 hours followed by a rebound increase at 48 hours was observed in treatments with high initial expression levels, namely 0.1 mM IPTG, 2.5% and 5% lactose, and 2.5% skim milk. This pattern indicates metabolic stress resulting from the production of large amounts of recombinant protein during the early induction phase. Excessive protein production requires high amounts of energy and biosynthetic precursors, which can disrupt cellular metabolic balance (Bhattacharya & Dubey, 1995; Rahmen *et al.*, 2015). Once the cells adapt to these conditions, expression capacity can increase again at longer induction times (Shiloach & Fass, 2005).

The decline in expression over 24 hours is also associated with the accumulation of metabolic byproducts and cellular stress responses. In high-density *E. coli* cultures, overflow metabolism produces acetate, which can inhibit cell growth and recombinant protein synthesis (Shiloach & Fass, 2005). Furthermore, large-scale protein production often leads to misfolding and aggregate formation, which triggers the activation of heat shock proteins and the protease system, thereby temporarily suppressing new protein synthesis (Hoffmann & Rinas, 2004; Hoseinpoor *et al.*, 2020). This condition reflects a cellular adaptation phase prior to the recovery of protein synthesis activity during the subsequent induction period.

Further Tukey HSD analysis showed that the 2.5% skim milk treatment consistently produced the highest expression intensity. The 5-hour induction time in this treatment was not significantly different from the 48-hour induction time, as both showed the same expression levels. These findings indicate that maximum expression was achieved during the early induction phase and could be maintained through longer induction periods, suggesting that short induction conditions have the potential to enhance process efficiency without a significant reduction in yield (You *et al.*, 2018).

The advantages of skim milk as an inducer are related to its composition and mechanism of action. IPTG is a synthetic inducer that is not metabolized, thereby triggering strong and constant induction; however, it has the potential to increase cellular stress (Donovan *et al.*, 1996; Myung *et al.*, 2020). Lactose is a natural inducer that is metabolized by cells, resulting in slower induction that can be influenced by catabolite repression (Seung-Hyun *et al.*, 2020; Vasconcelos *et al.*, 2018). Skim milk contains lactose along with additional nutrients such as protein, vitamins, and minerals that support cell growth and physiological stability during protein production. The combination of these components produces a more balanced and efficient induction effect compared to a single inducer (Greicius *et al.*, 2023; Pugliese *et al.*, 2017; Shah *et al.*, 2022).

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Overall, the results of this study indicate that the interaction between inducer concentration and induction time is a key factor in determining the success of recombinant GST-bLf CAR protein expression in *E. coli* BL21. Treatment with 2.5% skim milk and a 5-hour induction time was found to be the most optimal condition in this study. These findings provide a strong scientific basis for the development of more efficient, economical, and practical induction strategies in the production of the recombinant GST-bLf CAR protein.

CONCLUSIONS AND SUGGESTIONS

Conclusions

Based on the research results, it can be concluded that the type and concentration of the inducer, as well as the induction time, have a significant effect on the expression level of the recombinant GST-bLf CAR protein in *E. coli* BL21 ($p < 0.01$). Skim milk inducer demonstrated the most optimal induction capability, particularly at a concentration of 2.5%, and produced a significantly higher level of protein expression compared to 0.1 mM IPTG inducer or lactose at all tested concentrations. Additionally, the induction time factor plays a crucial role in determining the intensity of protein expression, showing significant differences in expression as the duration of induction increases. A significant interaction between inducer concentration and induction time indicates that the effectiveness of protein induction is highly dependent on the combination of these two factors. Overall, the treatment combination of 2.5% skim milk with a 5-hour induction time was the most optimal condition for producing the recombinant GST-bLf CAR protein compared to other treatment combinations.

Suggestions

Further research is needed to evaluate the quality of GST-bLf CAR recombinant proteins and optimize expression parameters to support the use of skimmed milk as an efficient and applicable alternative inductor.

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Tables

Table 1. Dot blot intensity values quantified using ImageJ for GST-bLf CAR protein expression in 48-hour cultures, observed 5 hours, 24 hours, and 48 hours after induction with 0.1 mM IPTG, 1%, 2.5%, and 5% lactose, and 1%, 2.5%, and 5% skim milk.

Induction Time	Repetition	Inductor							
		Control	IPTG	Lactose			Skim Milk		
				1%	2.5%	5%	1%	2.5%	5%
5	1	0.35	2.16	1.72	0.77	0.70	8.45	13.86	5.32
	2	0.30	2.43	1.87	0.86	0.80	9.54	14.31	5.99
	3	0.37	2.79	1.99	0.92	1.30	9.41	15.43	6.75
	Mean	0.34	2.46	1.86	0.85	0.93	9.13	14.53	6.02
	SD	0.04	0.32	0.14	0.08	0.32	0.60	0.32	0.72
24	1	0.58	1.89	0.84	0.88	1.21	5.46	8.02	7.05
	2	0.59	1.98	0.83	0.95	1.37	6.33	7.86	6.90
	3	0.66	1.96	0.92	1.13	2.13	6.34	9.01	7.57
	Mean	0.61	1.95	0.86	0.99	1.57	6.04	8.30	7.17
	SD	0.04	0.05	0.05	0.13	0.49	0.51	0.62	0.35
48	1	1.41	3.17	1.92	2.81	2.88	2.44	14.49	11.01
	2	1.44	3.25	1.95	2.77	2.87	2.51	14.51	11.23
	3	1.54	3.46	2.01	2.98	2.95	2.76	15.86	12.53
	Mean	1.46	3.29	1.96	2.85	2.90	2.57	14.95	11.59
	SD	0.07	0.15	0.05	0.11	0.04	0.17	0.79	0.26

Table 2. Results of the Shapiro–Wilk test for GST-bLf CAR protein expression levels across various treatment groups.

Treatment	Statistics	Significance (p>0.05)	Remarks
Negative Control	0.811	0.027	Abnormal
IPTG 0.1 mM	0.883	0.168	Normal
Lactose 1%	0.740	0.004	Abnormal
Lactose 2.5%	0.714	0.002	Abnormal
Lactose 5%	0.829	0.044	Normal
Skim milk 1%	0.882	0.166	Normal
Skim milk 2.5%	0.787	0.015	Abnormal
Skim milk 5%	0.861	0.099	Normal

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Table 3. Results of a two-way ANOVA analysis of the expression levels of the recombinant GST-bLf CAR protein based on variations in induction time and inducer concentration.

Sources of diversity	F value	Significance (p<0.01)
Inductor	985	0.000
Time	120	0.000
Time * Inductor	81	0.000

Table 4. Results of the Tukey HSD post-hoc test on the effect of variations in inducer concentration and induction time on the expression level of the recombinant GST-bLf CAR protein.

Inductor	Consentration	Time	Mean	SD	Notation
Control	–	5 hour	0.34	0.04	a
Control	–	24 hour	0.61	0.04	ab
Control	–	48 hour	1.46	0.04	abcd
IPTG	0.1 mM	5 hour	2.46	0.32	def
IPTG	0.1 mM	24 hour	1.95	0.05	cde
IPTG	0.1 mM	48 hour	3.29	0.15	f
Lactose	1%	5 hour	1.86	0.14	bcde
Lactose	1%	24 hour	0.86	0.05	abc
Lactose	1%	48 hour	1.96	0.06	cde
Lactose	2.5%	5 hour	0.85	0.08	abc
Lactose	2.5%	24 hour	0.99	0.13	abc
Lactose	2.5%	48 hour	2.85	0.11	ef
Lactose	5%	5 hour	0.86	0.32	abc
Lactose	5%	24 hour	1.39	0.49	abcd
Lactose	5%	48 hour	2.9	0.04	ef
Skim milk	1%	5 hour	9.13	0.60	i
Skim milk	1%	24 hour	6.04	0.51	g
Skim milk	1%	48 hour	2.57	0.17	def
Skim milk	2.5%	5 hour	14.53	0.32	k
Skim milk	2.5%	24 hour	8.3	0.62	hi
Skim milk	2.5%	48 hour	14.95	0.79	k
Skim milk	5%	5 hour	6.02	0.72	g
Skim milk	5%	24 hour	7.17	0.35	gh
Skim milk	5%	48 hour	11.59	0.26	j

Description of notation: Treatments with the same notation (p>0.05). Conversely, treatments with different notations are significantly different.

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Figures

Figure 1. Culture of *E. coli* BL21 bacteria containing the bLf CAR plasmid

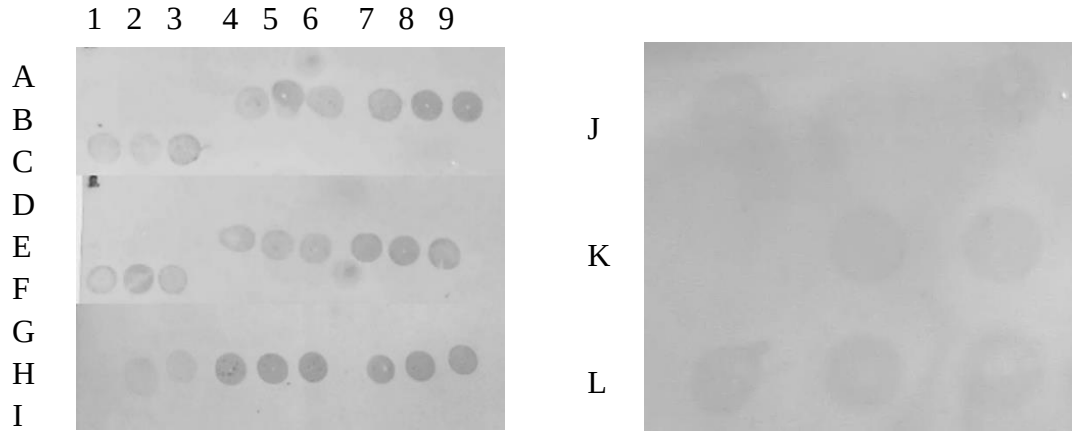

Figure 2. The left panel shows the results of dot blot induction assays using lactose, skim milk, and a negative control at induction times of 5 hours, 24 hours, and 48 hours. At the 5-hour induction time (rows A–C), 1%, 2.5%, and 5% lactose were located at A4–A6, A7–A9, and B1–B3, respectively, while 1%, 2.5%, and 5% skim milk are in B4–B6, B7–B9, and C1–C3, and the negative control is in C4–C6. In the 24-hour induction (rows D–F), 1%, 2.5%, and 5% lactose were in D4–D6, D7–D9, and E1–E3, while 1%, 2.5%, and 5% skim milk were in E4–E6, E7–E9, and F1–F3, and the negative control was in F4–F6. At the 48-hour induction (rows G–I), 1%, 2.5%, and 5% lactose were in G1–G3, G4–G6, and G7–G9, while 1%, 2.5%, and 5% skim milk were in H1–H3, H4–H6, and H7–H9, and the negative control was in I1–I3. The right panel shows the results of induction using 0.1 mM IPTG as a positive control at 5 hours (row J), 24 hours (row K), and 48 hours (row L), with each treatment represented by three dots indicating three biological replicates; all experiments in the left and right panels were conducted separately at different time points.

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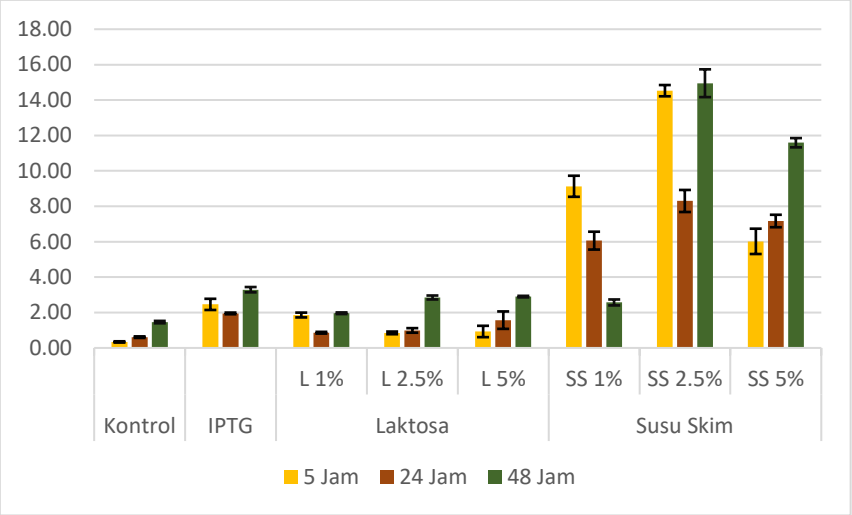

Figure 3. Graph showing the average dot blot intensity values measured using ImageJ for the expression of the recombinant GST-bLf CAR protein. The standard deviation is indicated by bars representing ±1 SD.

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