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TEMPERATURE EFFECTS ON JEMBRANA SUPERFICIAL UNIT (JSU) RECOMBINANT PROTEIN EXPRESSION IN *Escherichia Coli* BL21

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ABSTRACT

The efficiency of expression of recombinant proteins in host cells depends on achieving proper protein folding, solubility, and biological activity. Critical environmental factors, including temperature, pH, nutrient availability, and inducer concentration, significantly influence protein yield, stability, and functionality. Optimizing these conditions is essential to minimize protein misfolding and aggregation, which can adversely affect expression efficiency and reduce bioactivity. This study aims to measure the effect of temperature on the growth of *Escherichia coli* BL21 harboring the recombinant JSU protein, derived from the ENV-SU gene of the Jembrana virus. A controlled laboratory experiment was conducted in which bacterial cultures were incubated at 25°C and 37°C under identical conditions, except for temperature. The results indicate a statistically significant difference in bacterial growth between the two temperature conditions (p = 0.08), suggesting that *E. coli* BL21 exhibits enhanced growth at 37°C compared to 25°C. The mean bacterial growth rate at 37°C was 1.756, whereas at 25°C, the average growth was 1.425, demonstrating that higher temperatures facilitate bacterial proliferation. However, due to the relatively small sample size, further validation with a larger dataset is required to confirm these findings. These findings highlight the critical role of temperature optimization in enhancing bacterial growth and improving recombinant protein production efficiency, emphasizing the need for further investigation with a larger dataset to validate the results.

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Introduction

Recombinant protein expression in host cells aims to achieve proteins with optimal folding, solubility, and activity (Hashemzadeh et al., 2021; Nag et al., 2022; Stollar & Smith, 2020). One of the main factors that influence the success of recombinant protein expression is the composition and optimization of the environment during the production process (Beygmoradi et al., 2023). Environmental factors such as temperature, pH, nutrient availability, and inducer concentration are crucial in determining protein yield, solubility, and functionality. Optimizing these parameters effectively prevents protein misfolding and aggregation, which can result in low expression efficiency and decreased bioactivity (Zhang et al., 2022). Therefore, enhancing the production of recombinant proteins in different environments requires understanding how these parameters interact.

Escherichia coli is widely used as a host cell for recombinant protein expression. Protein recombinant expression often comes using *E. coli* BL21 and its derivatives (Robichon et al., 2011). However, temperature is a critical factor significantly influencing protein expression within host cells. Previous studies have shown that temperature impacts the growth dynamics and induces phenotypic alterations in *E. coli* colonies (Noor et al., 2013). Temperature variations also affect cellular physiology, including membrane fluidity and nucleic acid structure. These alterations may decrease the efficiency of RNA translation, transcription, and degradation, ultimately affecting overall cellular function (Volkandari & Margawati, 2020). Therefore, maintaining an optimal temperature is crucial to ensuring proper cellular processes.

Considering the important role of temperature in recombinant protein expression, it is necessary to understand its impact on the production of Jembrana Superficial Unit (JSU) in *Escherichia coli* BL21. This study aims to analyze how temperature variations affect JSU expression levels, providing insights into optimizing conditions for efficient recombinant protein production.

Materials and Methods

Material of this study

The recombinant protein used in this study was designed from the *ENV-SU* gene of the Jembrana virus encoding the JSU protein, inserted into an expression plasmid (pET21a), and transformed into *Escherichia coli* strain BL21 host cells (Volkandari & Margawati, 2020). *E. coli* BL21 containing JSU construct recombinant protein in glycerol stock. The Animal Molecular Genetics Laboratory, Biotechnology Research Center, and Indonesian Institute of Sciences provided the JSU construct. A total of 200 µL of *E. coli* BL21 glycerol stock (DE3) was inoculated into 5 ml of liquid LB medium containing 5 µl of ampicillin at a concentration of 100 µg/ml, followed by incubation of the culture in a shaking incubator (150 rpm, 37ºC) overnight.

Study design and variables

A controlled laboratory experiment was conducted to evaluate the effect of temperature on bacterial growth. The study involved two treatment groups, where bacterial cultures were incubated at 25°C and 37°C under identical conditions except for temperature. The dependent variable, bacterial growth rate, was measured by optical density (OD 600 nm) readings using a spectrophotometer. The sample consisted of six independent bacterial cultures, with three replicates per treatment group. The sample size selection was based on prior studies using t-tests to compare bacterial growth rates under different environmental conditions. A power analysis determined the minimum required sample size, with an alpha level of 0.10 and 80% study power.

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Induction of recombinant protein

A sample of 1 mL was measured for cell density (OD) at 600 nm using a spectrophotometer until it reached 0.4-0.6. Afterwards, induction was carried out by adding 50 µL of IPTG with a concentration of 0.6 µM. A total of 6 ml of culture samples were incubated using a shaker incubator at 37ºC and 25ºC with a rotation of 150 rpm for 1 hour. 14 ml of culture from the incubation was poured into a centrifuge tube. Centrifugation was carried out with a rotation speed of 4,000 rpm at 10ºC for 20 minutes. The supernatant and pellet obtained from the centrifugation results were then separated. The pellet was collected and washed using cold PBS. Culture samples were then re-incubated using a shaker incubator at 25°C and 37°C, rotation speed of 150 rpm for 1 hour. Centrifugation was performed again to obtain the 2nd pellet collection with a rotation speed of 4,000 rpm at 10ºC for 20 minutes. The pellets obtained were then collected and stored in a freezer at -20ºC.

Cell lysis and quantification of protein

The freeze-thaw method was used for cell lysis. Pellets stored at -20°C were thawed at room temperature, then mixed with 2 mL of lysis buffer. The samples were refrozen and subjected to three freeze-thaw cycles. After the final cycle, 1 mL of dH_2O was added. A 20 µL aliquot of the lysed protein sample was transferred to a mini tube and labeled. Quantification was performed using a Gene Quant machine. 36 recombinant protein samples were centrifuged for 20 minutes, and the results were supernatant and pellet. The supernatant was then collected and put into a minitube to be quantified using a spectrophotometer with a wavelength of 600 nm. The centrifuged pellet was then stored in the centrifuge freezer at -20 C. The blank solution was then put into a cuvette. One µl of supernatant from the protein sample was put into the cuvette. The cuvette containing the protein sample was then inserted into the Gene Quant machine to calculate the quantification value by pressing the protein culture button, and the quantification value appeared on the monitor.

Data Collection Procedure

Cell density measurement in this study began with rejuvenating *E. coli* BL21 cells carrying the recombinant protein JSU construct in a Luria Bertani (LB) medium. One factor that can influence the growth of *E. coli* BL21 is temperature. The incubation temperature of *E. coli* BL21 cells before induction ranges from 37–41°C, which is the optimal growth temperature for *E. coli* in general (Abuei et al., 2022; Okik Hendriyanto, 2010). This study quantified protein samples using a Gene Quant machine and a spectrophotometer. The table below presents the quantification results obtained using a spectrophotometer at a wavelength of 600 nm.

Statistical analysis

All statistical analyses were performed using R software. Before conducting the t-test, the data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Since both assumptions were met (p>0.05 for normality and variance homogeneity), an independent samples t-test (Student's t-test) was performed to compare bacterial growth rates at 25°C and 37°C. The test was one-tailed, with the alternative hypothesis (H_1) stating that bacterial growth at 37°C was significantly higher than at 25°C.

Results and Discussion

This study began by measuring the cell density of *Escherichia coli* BL21 carrying the recombinant protein construct JSU. The cell revitalisation process originated from an 80% glycerol stock cultured in a Luria-Bertani (LB) medium to ensure optimal protein expression quality and quantity. The

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LB medium contained 100 µg/mL ampicillin as a selective marker, allowing only the growth of recombinant *E. coli* BL21 harboring the pBT7-C-His plasmid. Ampicillin is a β-lactam antibiotic that disrupts bacterial cell wall synthesis by targeting penicillin-binding proteins (Good, 2008). This action prevents the growth of non-resistant bacterial cells. It aligns with a previous study; we use ampicillin as a selective marker to ensure that only *E. coli* BL21 cells carrying the pBT7-C-His plasmid, which provides resistance to ampicillin, can grow. This process helps maintain plasmid stability and increases the expression of recombinant proteins (O'Brien et al., 2021; Sadegh Feizollahzadeh, 2017; Yu et al., 2024).

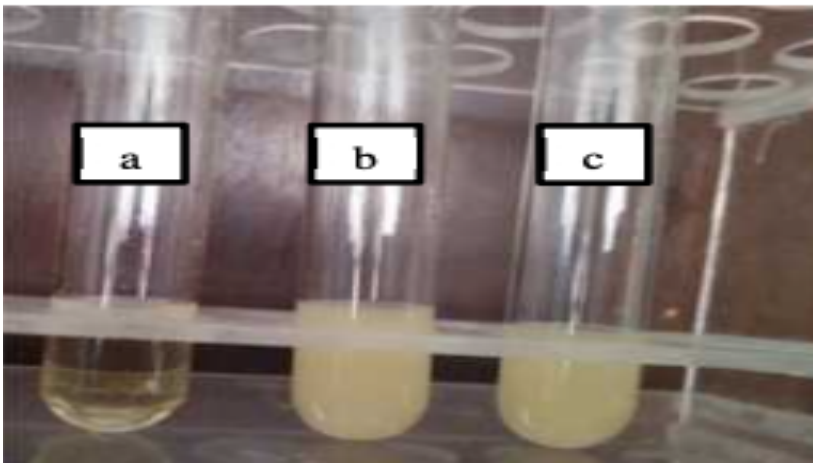

Figure 1. Culture of *E. coli* BL21 on LB media containing 100 µg/ml Ampicillin (a) LB media without *E. coli* BL21 isolate (b) culture of *E. coli* BL21 (c) culture of *E. coli* BL21.

The LB medium supplemented with 100 µg/mL ampicillin resulted in a higher cell density of recombinant *E. coli* BL21 than the medium without ampicillin. This increased cell density directly impacts recombinant protein production, which is influenced by several factors, including temperature, incubation time, and growth medium composition(Modi et al., 2023; Shahzadi et al., 2021). The optimal temperature for the growth of *E. coli* BL21 before induction is between 37°C and 41°C (Ceylan, 2023; Okik Hendriyanto, 2010; Shariati et al., 2021). Additionally, incubation time plays a crucial role, as longer incubation periods result in higher cell density, enhancing recombinant protein production (Ganjave et al., 2022; HERMANA, 2015)

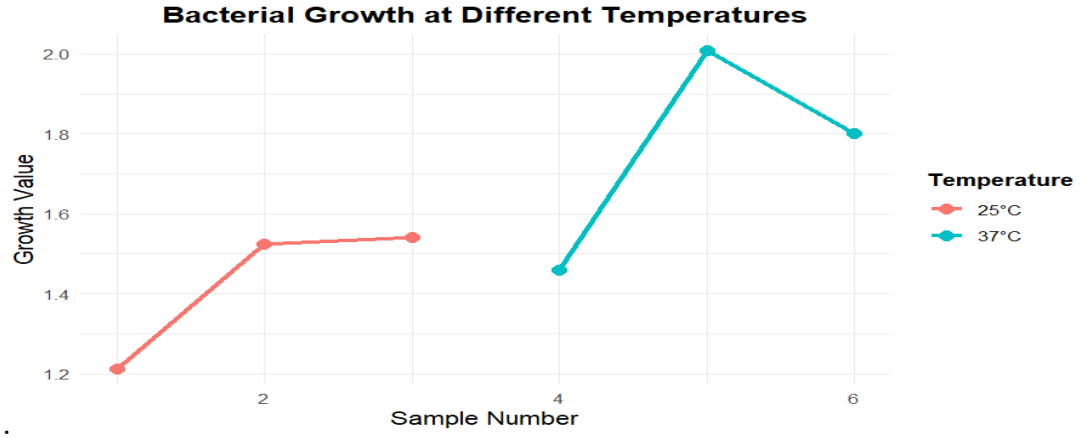

Figure 2. Bacterial Growth in different temperatures (37ºC and 25ºC).

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Our study found that the t-value from the test was 1.718, with 4 degrees of freedom (Figure 2), indicating a moderate difference in bacterial growth between the two temperature conditions. The p-value of 0.08 is slightly below the chosen significance level of $\alpha = 0.10$, providing sufficient evidence to reject the null hypothesis at the 10% significance level. This suggests that recombinant protein expression at 37°C is significantly higher than at 25°C. These findings align with previous research demonstrating that 37°C is the optimal temperature for bacterial growth and protein synthesis (Jiang et al., 2022; Laporte et al., 2021).

Temperature is a critical factor influencing bacterial growth and metabolic activity. When exposed to temperatures below their minimum threshold or slightly above their maximum tolerance, bacteria enter a dormant state, leading to reduced metabolic function and slower growth. The optimal temperature range for bacterial proliferation is generally between 8°C and 46°C, with 37°C being the most favorable for many species (Melliawati, 2009; Volkandari & Margawati, 2020). This is particularly relevant for recombinant protein production, where maintaining optimal incubation conditions can enhance yield and efficiency. Understanding these temperature effects is essential for applications in biotechnology, including industrial fermentation and pharmaceutical manufacturing

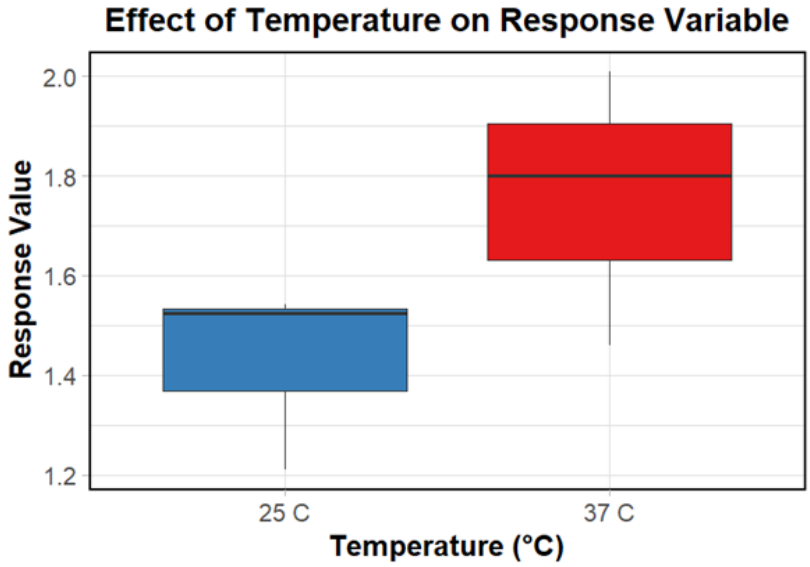

Figure 3. Effect of temperature on growth rate of *E. coli* BL21 containing JSU construct.

The mean bacterial growth rate at 37°C was 1.756, while at 25°C, it was 1.4253 **(Figure 3)**. The higher mean value at 37°C aligns with expectations that bacteria grow more rapidly at optimal physiological temperatures. However, due to the relatively small sample size, the variability within the data might influence the results, requiring further validation with a larger dataset (Andrade, 2020; Hennink & Kaiser, 2022; Lakens, 2022). Therefore, further validation with a larger dataset must confirm these findings and ensure their generalizability.

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Conclusions

This study suggests that temperature plays an essential role in the growth of *Escherichia coli* BL21 containing recombinant JSU protein, with higher bacterial proliferation observed at 37°C compared to 25°C. Statistical analysis confirmed the significant difference in growth rates, indicating that 37°C provides a more favorable environment for bacterial replication, thereby enhancing recombinant protein production. These findings highlight the importance of temperature optimization in microbial expression systems to maximize protein yield and stability. Additionally, optimized microbial expression systems in environmental and industrial biotechnology can contribute to developing sustainable bio-based solutions, including enzyme production and bioremediation efforts. Further research with larger datasets is necessary to validate these findings and explore additional parameters influencing recombinant protein expression across diverse applications.

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