Effect of storage time and temperatures on growth rate of
Eschericia coli BL21 containing JSU construct for Jembrana
disease candidate vaccine

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Abstract. The viability of Eschericia coli BL21 (DE3) bearing JSU (Jembrana Surface Unit) recombinant protein depend on several factors, such as temperatures and time of storage. JSU act as a vaccine candidate for the Jembrana disease in Bali cattle, therefore, it is important to keep the quality and quantity the yield of JSU recombinant protein, the E. coli need to maintenance. The study was designed to examine the viability of Escherichia coli at different temperatures and time of cultivation. The E. coli BL21 bearing JSU was cultured in LB broth (+Amph 100µg mL⁻¹) overnight and aliquot it in 1.5mL sterile tube (3 tube every temperatures). The tubes were incubated in three storage temperatures (Room Temperatures/RT, 4°C and -20°C. The viabilities of the cells were observed in 24, 48 and 120 hours by measuring the Optical density by spectrophotometer, and then inoculated it in LB plate (+Amp 100µg mL⁻¹). The result showed that statistically, there were significant effect (P<0.05) in temperature -20°C at 24h and 120h observation on viability of E. coli BL21. The viability of E. coli at -20°C and storage time observed at 120h are more stabile, compared the other temperatures (RT and 4°C). There was no significant effect on both temperatures RT and 4°C in OD₆₀₀ at 24 and 48h and the growth was decreased until 120h observation. The observation at 24h with 4°C cultured has a higher OD₆₀₀ (1.098) followed by RT (1.070) and -20°C (0.773). The viability of E. coli was decreased at 48h with RT (1.011) and 4°C (1.0611), meanwhile, at -20°C the cells were increased (0.958). Meanwhile, the observation at 120h, the cells was more stabile at -20°C (0.961) compared with cultures from 4°C (0.981) and RT (0.719). Based on the OD₆₀₀, the viability at the -20°C intended more stabile compared to RT and 4°C, but in the 24h the growth at -20°C are very low because no protectant added in the culture, it will shock the cells and dead. This finding showed that viability of E. coli BL21 bearing JSU recombinant protein better to keep in -20°C for longer period storage time with an addition of protectant agent in the culture media to keep the cells alive and more stabile.

1. Introduction
Escherichia coli is a bacteria that is widely used as a host for the expression and production of recombinant proteins. The advantages of E. coli for expression are high protein yields, low cost production, grow rapidly and easy transformation [1] [2]. The disadvantage of E. coli as a host cell is to produce the recombinant protein, it will need to maintain to keep the bacteria and plasmid alive, also to keep the quality and quantity of recombinant protein expression in an optimal condition.
The applications of recombinant protein technology make it possible in assembling veterinary vaccines originating from the gene of virus genome. Recombinant Protein was used in this study was designed from env-su Jembrana virus gene encoded JSU protein, which is inserted into the plasmid expression (pET21a) and transformed into Escherichia coli strain BL21 host cell to produce the vaccine [3]. The E. coli BL21 and derivatives are common for protein recombinant expression [4], commented, that strain BL21 (DE3) contains a chromosomal copy of the T7 RNA polymerase gene for simple and efficient expression of genes under control of the T7 promoter [5]. JSU protein has the function of mediating the entry of viruses into target cells and is very potential as a vaccine because it can block the introduction of receptors so that it can prevent the entry of viruses into cells [6].

There was environmental factor that affected the E. coli viability, need to be consideration such as, temperature, and dealing with temperature change is crucial for adaptation [7]. Previously studied showed that the nutrition in the medium culture and temperatures will affect in growth and change of E. coli colonies phenotype [8]. The temperature also changes the cellular physiology such as membrane fluidity and structure of nucleic acids, it will reduces the efficiency of RNA translations, transcriptions and degradations [9].

Based on previous study, the current research was designed to find the optimal condition to maintain the E. coli cells bring the JSU recombinant protein to keep the quality and quantity of JSU recombinant protein expression by applying the combination between storage time and temperatures with no cryoprotectant (glycerol) added into medium culture for short term storage.

2. Method
2.1 Materials.
*Eschericia coli* strain BL21 (DE3) bearing JSU construct protein recombinant in a glycerc stock. The JSU construct belongs to Animal Molecular Genetics Laboratory-RC for Biotechnology LIPI.

2.2 Overnight culture.
An amount 40µL of *E. coli* BL21 (DE3) gliserol stock were inoculated into 10 mL of Luria Bertani (LB) media containing 100µg mL⁻¹ Ampicillin, then the culture was incubated in shaker incubator (150rpm;37°C) overnight.

2.3 Temperature and time storage effect.
Aliquots (10ml) of the overnight culture were distributed into 18 sterile, 1,5-mL tubes. Sets of three tubes were independently incubated at Room Temperature/RT, 4 and -20°C. One tube from each temperature treatment incubated at 24, 48, and 120 h [10] and the viability of *E. coli* were analyzed by cultivated the colonies into LB plate (+100µg mL⁻¹ AMP), then the plates were incubated into 37°C for overnight. The colonies were grown observed and counted.

2.4 Statistical Analysis.
The optical density (OD₆₀₀) of all the grown cultures were measured every 24h, 48h and 120h and the result were analyses by Analysis of Variance and followed by Duncan Test.

3. Result and Discussions
The result of this study was analysed, that the viability of *E. coli* BL21 cell depending on temperature and period time of storage where the cells are kept. Based on the analysis, that those factors are had significant effect on the living on *E. coli* cells. The analysis was shown in Table 1.

| Treatments | Time cultivation (h) | Total |
|------------|----------------------|-------|

Table 1. Statistical analysis of viability of *E. coli* BL21 cell against temperature and time storage
Temperature (°C) 24 48 120
Room Temperature (RT) 1.070 ±0.142 1.011 ±0.009 0.719 ±0.204 0.934±0.204
4°C 1.098 ±0.012 1.061 ±0.008 0.987 ±0.043 1.045±0.053
-20°C 0.773 ±0.064 0.958 ±0.047 0.961 ±0.005 0.898±0.101
Total 0.9809±0.174a 1.007±0.047a 0.889±0.164b

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a,b Superscript in the different column and row have a significant effect (P<0.05)

The analysis in Table 1 shown, that the treatment has significant effect on viability of E. coli, but at -20°C the growth of E. coli almost similar from 24h until 120h observation. This is because, in the low temperature the E. coli in the dormancy position, but when the E. coli were cultivated in LB plate, there were no any colonies grown in 120h observation. At low temperature, the fluidity of membrane will decreases and it permeability will increased [7] [11]. The cells become adapted to low temperature and resume growth but at a slower rate the expression of the cold-inducible proteins declines [9]. In this study, the E. coli cell not growth after 120h (figure 2), due to the absence of cryoprotectant material on the storage media.

The research for effect of storage temperature (at 4, 10, and 21.1 °C) and the time (from 0 to 365 days) on the survival of the inoculated organisms was evaluated at different sampling times (0, 30, 60, 120,180, and 365 days) was done by Adhikari et al (2018) [12], also had a significant between treatment on the viability of the test organisms. The viability of E. coli also inhibited when temperature increasing [8].

Eschericia coli from each treatment was cultivated onto LB media (+Amp), the result from 24h and 48h observation the E. coli grow finely (see Fig. 1 and 2), but at 120h observation (see Fig. 3), the colony from -20h nothing showed no any colony grew on the LB media plate. This phenomenon seems contrast with the reading of OD$_{600}$ of the E. coli cells for 120h cultivation (Table 1).

Fig. 1 The performance of E. coli cells at 24h of cultivation

Fig. 2 The performance of E. coli cells at 48h of cultivation

Fig. 3 The performance of E. coli cells at 120h of cultivation
The colony of *E. coli* in LB plate still grow at 24h observation in all temperatures (Figure 1), this result was mentioned [13], the right temperature for bacterial growth is between 8°C-46°C and optimum at 37°C, while temperatures below the minimum temperature or slightly higher than the maximum temperature will slow bacterial growth because the bacteria experience dormancy or sleep.

Based on Figure 3, it can be seen that *E. coli* strain BL21 bearing JSU recombinant was successful grew in LB plate for treatment stored at RT (Room Temperature) and 4°C while at temperatures of -20°C at 120h the *E. coli* colonies was death, so there were no any colony grew on the plate. This can be happened because the LB media culture was not mixed with glycerol when it stored in temperatures at -20°C.

Glycerol functions as a protective layer for bacteria when it is stored at a minus temperature, so that bacteria can sleep in a coated state and not death. Glycerol has a high solubility with liquid triptic soy broth media even in cold conditions, has the ability to penetrate into the cell and has a low toxicity [14]. Glycerol is also able to prevent the collection of water molecules and the crystallization of ice at the freezing point of the solutions, glycerol will also modify ice crystals formed in the freezing medium to inhibit cell damage mechanically [15].

4. Conclusions
Based on statistical analysis, there were significant effect on temperatures and storage time for viability of *E. coli*. The lowest temperature in the long period storage will gave high viability on *E. coli* cells. But, in the long term of storage, the *E. coli* cells need to keep in the medium with glycerol adding as a cryoprotectant to maintain the viability of the cells.

5. Acknowledgement
Author thanks to Kurniawan and Ade Irma Sari Br. Maha (UNNES student) for great assistance in the Laboratorium of Animal Molecular Genetic Research Center for Biotechnology –LIPI.

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