Comparison of Cultured S.Aureus and E.coli DNA Concentrations on Growth Media of Luria Bertani and Nutrient Broth

Trisiswanti[1,*] Anggi Maulia Arista[1], Eza Alfian Rizqita[1], Sugimin[1], Rizki Yulia Oxi[1]

[1] *Universitas Negeri Surabaya, Surabaya, Indonesia*
**Corresponding author Email: trisiswantitrisiswanti@unesa.ac.iD*

ABSTRACT
This study aimed to describe the comparison of cultured S.aureus and *E.coli* DNA concentration on growth media of Luria bertani and Nutrient broth. S.aureus and *E.coli* bacteria that have been cultivated on Luria Bertani (LB) and Nutrient Broth (NB) media, after measuring their absorbance values at a wavelength of 620 nm, have quite high absorbance values. For *S. aureus* bacteria which are gram-positive bacteria, Luria Bertani (LB) media is the best medium for cultivation, because the absorbance value is 1.324 ± 0.500, while *S. aureus* cultivated on Nutrient Broth (NB) growth media has a lower absorbance value. ie 1.047 ± 0.500 which means the highest cell density or optical density (OD) for S.aureus was cultivated on Luria Bertani (LB). The highest DNA concentration was found in NB *E.coli* isolates at 9.50 ng/µl. The best level of DNA purity was found in the isolates of NB S.aureus, which was 1.89 based on the A value of 260/280, while for the isolates LB *E.coli*, NB *E.coli*, and LB S.aureus were also good, but there were few contaminants so that A score of 260/280 produces a value below 1.8 and more than 2.0.

Keywords: *DNA, cultivated, culture media, Staphylococcus aureus, Escherichia coli*

1. INTRODUCTION

Escherichia coli bacteria are normal bacteria in the intestines, but in abnormal numbers become pathogenic bacteria [1]. These bacteria are the cause of acute diarrhea at all ages. These bacteria stick to the intestines and produce toxins that can damage the mucosal cells of the small intestine [2]. *Staphylococcus aureus* is a Gram-positive bacterium that often infects humans and animals [3]. This bacterium is a bacterium that causes infection in general which always shows increased resistance to various types of antimicrobials [4].

Luria Bertani media is often used for the cultivation of *Escherichia coli* bacteria and other microorganisms that are not too difficult to culture. This medium is often used for genetic and molecular studies of microorganisms. Luria farming is also a medium rich in nutrients such as peptone, nitrogen compounds, amino acids, vitamins, sodium chloride, and other growth factors needed by microorganisms [5]. Nutrient Broth (NB) is a universal medium used to grow bacteria in general [6].

Molecular level analysis with DNA as the object begins with extraction. The DNA extraction process is to obtain pure DNA with a high concentration so that it can be used for further molecular analysis such as PCR, RLFP, and RAPD[7] [8]. Good extraction is supported by the results of the quantity of DNA extract obtained. 260 and 280 nm waves. DNA purity was determined by calculating the ratio of absorbance at A260 and A280. A DNA molecule is said to be pure if its absorbance ratio ranges from 1.8 to 2.0 [9].

Information on the concentration and purity of *S.aureus* and *E.coli* DNA that was cultivated on the growth media of farmed Luria and nutrient broth is very necessary to determine the degree of contamination and whether the sample is good for use in the next stage. Therefore, measurements were made on the quantity, concentration, and purity of genomic DNA.

2. MATERIAL AND METHODS

The number of bacterial cells that have been cultivated on growth media, then measured Optical Density (OD) using a Spectrophotometer-UV-Vis at a certain wavelength.[10] in their research measured the optical density of Escherichia coli at the same wavelength of 600 nm. Different wavelengths used to measure Optical Density (OD) were carried out by [7]

for Pseudomonas aeruginosa and Staphylococcus aureus, namely 492 nm. The DNA isolation process is a process to remove DNA from the cell mass and other components in the cell [3].

The quality of isolated DNA can be seen from the level of purity and concentration. DNA purity can be measured at wavelengths of 260 nm and 280 nm and DNA is said to be pure if the A260/A280 value is 1.8 - 2.0. The degree of contaminants is a factor that can affect the purity of DNA. If the value obtained is below 1.8, it indicates DNA contamination with protein or contaminants with phenol [8].

The calculation of DNA concentration can also be done using the NanoDrop tool. [3] in their research used Nanodrop to measure DNA concentration by dripping 1µl of sample on a NanoDrop device that was connected directly to a computer.

Tools and materials are sterilized first. All materials and tools were put into an autoclave and sterilized at 121ºC for 15 minutes with a pressure of 1 atm. Preparation of Luria Bertani Broth, Miller (LB) media by suspending 25 grams of LB media (Appendix 1) in 1 liter of distilled water which was homogenized by heating. The homogenized LB media was sterilized for 15 minutes by autoclaving at 121ºC, pressure 1 atm.

Bacterial isolates of *S. aureus* and E. Coli were recultured by inoculating one full ose into the Nutrient Agar (NA) medium. The bacterial isolates were then incubated for ± 24 hours at a temperature of 25ºC-30ºC until colonies were formed.

Bacteria that had been grown in Nutrient Agar (NA) media were re-grown in 1 ml on LB and NB media, then incubated for 24 hours at 30ºC. The incubated cultures were then completely re-grown on 5 ml of LB and NB media and incubated for 24 hours. The incubated cultures were then analyzed using spectrophotometry with a wavelength of 600-620 nm.

The bacteria that had been grown in LB and NB media were then centrifuged in a 500 l tube at a speed of 10,000 g for 1 minute. The supernatant was discarded, then the cell pellet underwent several steps such as cell resuspension with the addition of *resuspension Buffer*(Gram-positive), Cell lysis, Column Activation (Optional), Column loading, Primary washing, Secondary washing, and DNA elution by adding the Elution Buffer to the center of the column. The DNA formed was stored at 4°C or -20°C.

Bacterial DNA samples were calculated for concentration and purity using a spectrophotometer. DNA samples were measured at wavelengths of 260 nm and 280 nm. The level of DNA purity is calculated from the ratio of the wavelength of A260 to A280 (A260/280). Purity values for DNA that were categorized as pure ranged from 1.8 to 2.0.

This research is observational. The data from DNA isolation using the Kit method from Jena Bioscience (2021) was then quantitatively tested using a spectrophotometer with 3 replications. The data was obtained in the form of absorption (absorbance) of DNA at a wavelength of 260 nm and 280 nm.

3. RESULT AND DISCUSSION

Based on the research that has been done, data is obtained in the form of absorbance values at a wavelength of 620 nm. The absorbance value was used to analyze the bacterial cell density or Optical Density (OD) of two different growth media, namely Luria Bertani (LB) and Nutrient Broth (NB) media. Other data is in the form of DNA isolated from *S. aureus* and *E. coli* bacteria using the Kit from Jena Bioscience. The isolated DNA was then analyzed quantitatively using Nanodrop to determine the concentration and purity of the isolated DNA.

Cell density or Optical Density (OD) using Spectrophotometry-UV Vis will produce data as shown in Table 5.1.

Table 5.1 Results of Absorbance Measurement of Optical Density (OD)620 S.aureus and *E.coli* values on LB and NB Media.

MEDIA	AVERAGE ABSORBANCE OD_{620} ± SD
LBK	0,000 ± 0,000
LBSA	1,324 ± 0,500
LBEC	1,370 ± 0,500
NBK	0,000 ± 0,000
NBSA	1,047 ± 0,500
NBEC	1,382 ± 0,000

Information:
LBK : Media Luria Bertani as control
LBSA: Luria Bertani Media with *S. aureus* bacteria culture
LBEC: Luria Farming Media with *E.coli* bacteria culture
NBK: Media Nutrient Broth as control
NBSA: Nutrient Broth Media with *S. aureus* bacteria culture
NBEC: Nutrient Broth Media with *E.coli* bacteria culture

In general, bacterial growth media can be divided into two types, namely universal growth media and selective growth media. The universal bacterial growth media that are often used in the molecular analysis are Luria Bertani (LB) and Nutrient Broth (NB) media. Luria farming media (LB) is a medium consisting of various nutrients such as peptone, nitrogen compounds, amino acids, vitamins, and sodium [5]. Nutrient Broth (NB) media is also a universal medium that is often used for the cultivation of various types of bacteria. Nutrient Broth (NB) media consists of beef extract as a carbon source and peptone as nitrogen [6].

S.aureus and *E.coli* bacteria that have been cultivated on Luria Bertani (LB) and Nutrient Broth (NB) media, after measuring their absorbance values at a wavelength of 620 nm, have quite high absorbance values. For *S. aureus* bacteria which are gram-positive bacteria, Luria Bertani (LB) media is the best medium for cultivation, because the absorbance value is 1.324 ± 0.500, while *S. aureus* cultivated on Nutrient Broth (NB)

growth media has a lower absorbance value. ie 1.047 ± 0.500 which means the highest cell density or optical density (OD) for *S.aureus* was cultivated on Luria Bertani (LB) media. For *E.coli* bacteria which are gram-negative bacteria, Nutrient Broth (NB) media is the best medium for cultivation. The *E.coli* test bacteria used in the analysis were at least 108 cfu/ml or about 0.138 absorbance value at a wavelength of 620 nm. The data shows that *E.coli* cultivated with Nutrient Broth (NB) media has a higher absorbance value than *E.coli* cultivated on Luria Bertani (LB) media with a good Standard Deviation (SD) value of 1.382 ± 0.000 which means there is no difference after repeated measurements.

S.aureus and *E.coli* bacteria whose average absorbance value of OD620, had been measured were DNA isolated using the Kit from Jena Bioscience. DNA isolation using Kit is an alternative that is often used in molecular analysis. This method has several advantages such as easy to use and fast results. The steps in DNA isolation consist of cell isolation, cell wall and membrane lysis, extraction in solution, purification, and precipitation.

S. aureus and *E. coli* bacteria that had been grown on Luria Bertani (LB) and Nutrient Broth (NB) media were then isolated to obtain their DNA. The DNA sample is then tested quantitatively using Nanodrop which will produce data as shown in Table 5.2.

Table 5.2 Quantitative DNA Test Results

No.	Sample	Concentration (ng/µl)	A 260	A 280	A 260/280
1.	NBEC	9,50	0,190	0,118	1,61
2.	LBEC	4,50	0,089	0,039	2,27
3.	NBSA	2,70	0,054	0,029	1,89
4.	LBSA	-0,20	-0,004	-0,003	1,58

Information:
LBSA: Luria Bertani Media with *S. aureus* bacteria culture
LBEC: Luria Farming Media with *E.coli* bacteria culture
NBSA: Nutrient Broth Media with *S. aureus* bacteria culture
NBEC: Nutrient Broth Media with *E.coli* bacteria culture

The highest DNA concentration was found in NB *E.coli* isolates at 9.50 ng/µl. The best level of DNA purity was found in the isolates of NB S.aureus, which was 1.89 based on the A value of 260/280, while for the isolates LB *E.coli*, NB *E.coli*, and LB S.aureus were also good, but there were few contaminants so that A score of 260/280 produces a value below 1.8 and more than 2.0.

DNA of *S. aureus* and E. coli bacteria that have been isolated and then analyzed for concentration and purity. The highest concentration of DNA was found in E. coli DNA which was cultivated on Nutrient Broth (NB) media with a concentration of 9.50 ng/µl, and the lowest concentration of isolated DNA was found in *S. aureus* DNA cultivated on Luria Bertani (LB) medium with a concentration of -0.20 ng/µl, which is considered as no DNA has been isolated from the medium. The absence of DNA from *S. aureus* bacteria can be caused by various factors, one of which is the Kit method used in DNA isolation. [10]-[14] states that DNA isolation using a kit cannot guarantee the DNA isolation process can be successful.

The value of the purity of the isolation of *E.coli* DNA on Nutrient Broth (NB) 1.61 and Luria Bertani (LB) 2.27 media was not good. The same thing also happened to the purity value of *S. aureus* DNA isolation in Luria Bertani (LB) 1.58 media, while the purity value of *S. aureus* DNA isolation in Nutrient Broth (NB) 1.8 media had a fairly good and decent quality. to be used in further analysis. According to Septiani and Pendrianto (2018), DNA has good quality if the purity value of bacterial DNA obtained is high, ranging from 1.75 to 1.9. The low value of purity (<1.75) indicates that it is contaminated by the presence of protein, whereas if the value of the purity of bacterial DNA obtained is more than 1.9, it indicates that the DNA isolation results are contaminated with organic compounds, such as ethanol[15]-[16].

4. CONCLUSION.

The highest DNA concentration was found in NB *E.coli* isolates at 9.50 ng/µl. The best level of DNA purity was found in the isolates of NB S.aureus, which was 1.89 based on the A value of 260/280, while for the isolates LB *E.coli*, NB *E.coli* and LB S.aureus were also good, but there were few contaminants so that A score of 260/280 produces a value below 1.8 and more than 2.0.

ACKNOWLEDGEMENTS:

This project is sponsored by the LPPM University State of Surabaya.

REFERENCES

[1] Dima, L.L.R.H., Fatmawati, dan Lolo, W.A. 2016. Uji Aktivitas Antibakteri Ekstrak Daun Kelor (Moringa Oleifera L.) Terhadap Bakteri *Escherichia coli* Dan *Staphylococcus aureus. Pharmacon Jurnal Ilmiah Farmasi- Unsrat.* 2 (5): 2302- 2493.

[2] Tuntun, Maria. 2016. Uji Efektivitas Ekstrak Daun Pepaya (Carica Papaya L.) Terhadap Pertumbuhan Bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Jurnal Kesehatan*. 3 (VII): 497-502.

[3] Gardenia, L., dan Koesharyani, I. 2011. Metode Isolasi Deoxyribo Nucleic Acid Bakteri

Dari Organ Ikan Nila (*Oreochromis niloticus*) Untuk Diagnosa Streptococcociasis Dengan Teknik Polymerase Chain Reaction. *J. Ris. Akuakultur*. 3 (6): 469-477.

[4] Chen, C.J., dan Huang, Y.C. 2014. New Epidemiology of Staphylococcus Infection in Asia. *Clinical Microbiology and Infection*, 7 (20): 605-624.

[5] Himedia. 2016. *Luria Bertani Broth*, Miller (DM410). India: HiMedia Laboratories.

[6] Wahyuningsih, N., dan Zulaika, E. 2018. Perbandingan Pertumbuhan Bakteri Selulolitik Pada Media Nutrient Broth dan Carboxy Methyl Cellulose. *Jurnal Sains dan Seni ITS.* 2 (7): 2337-3520.

[7] Wijesinghe, G., Dilhari, A., Gayani, B., Kottegoda, N., Samaranayake, L., dan Weerasekera, M. 2019. Influence of Laboratory Culture Media on in vitro Growth, Adhesion, and Biofilm Formation of *Pseudomonas aeruginosa* and *Staphylococcus aureus. Med Princ Prac.* 28: 28-35.

[8] Fatchiyah, Arumningtiyas, E.L., Widyarti, S., dan Rahayu, S. 2011. *Biologi Molekular Prinsip Dasar Analisis.* Jakarta: Erlangga.

[9] Mustafa,H , Indra Rachmawati , Yusran Udin.2016. *Pengukuran Konsentrasi dan Kemurnian DNA Genom Nyamuk*. Jurnal Vektor Penyakit. 1 (10) : 7 - 10

[10] Low, S.X.Z., Loo, B.Z.L., Lee, K.C., Oon, J.S.H., Lee, C.H., Ling, M.H.T. 2013. Viability of Escherichia coli ATCC 8739 in Nutrient Broth, Luria-Bertani Broth and Brain Heart Infusion over 11 Weeks. *Electronic Physician*. 1 (5): 576- 581.

[11] Sun, D., Hale, L., dan Crowley, D. 2016. Nutrient Supplementation Of Pinewood Biochar For Use As A Bacterial Inoculum Carrier. *Biol Fertil Soil*. 52: 515-522.

[12] Wahyuningsih, N., dan Zulaika, E. 2018. Perbandingan Pertumbuhan Bakteri Selulolitik Pada Media Nutrient Broth dan Carboxy Methyl Cellulose. *Jurnal Sains dan Seni ITS.* 2 (7): 2337-3520.

[13] Puspitasari, D., Pramono, H., Oedjijono. 2014. Identifikasi Bakteri Pengoksidasi Besi Dan Sulfur Berdasarkan Gen 16s Rrna Dari Lahan Tambang Timah Di Belitung. *Scripta Biologica.* 1 (1): 8-14.

[14] Lee, K.E., Adhikari, A., Kang, S.M., You, Y.H., Joo, G.J., Kim, J.H., Kim, S.J., dan Lee, I.J. 2019. Isolation and Characterization of the High Silicate and Phosphate Solubilizing Novel Strain *Enterobacter ludwigii* GAK2 that Promotes Growth in Rice Plants. Agronomy. 9 (144): 1-12.

[15] Fitriya, R.T., Ibrahim, M., Lisdiana, L. 2015. Keefektifan Metode Isolasi DNA Kit dan CTAB/NaCl yang Dimodifikasi pada *Staphylococcus aureus* dan *Shigella dysentriae. Lentera Bio.* 1 (4): 87- 92.

[16] Pambudiono, A., Suarsini, E., Amin, M. 2016. Isolasi Dna Genom Bakteri Potensial Pengkelat Logam Berat Kadmium Dari Limbah Cair Penepungan Agar. *Seminar Nasional Pendidikan dan Saintek.* 103-107.