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Effectiveness of Bacterial Culture Preservative Techniques in the Microbiology Laboratory

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ABSTRACT

Short-term or long-term preservation goals are adjusted to program objectives to be designed. Various kinds of studies or research are carried out in the Laboratory Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, University State of Surabaya part great use isolate microbes as material the test. Bacterial culture storage space (refrigerator) is limited, so it is important for done study for find method which appropriate storage bacteria Escherichia coli and Staphylococcus aureus in glycerol medium. Isolate S. aureus and E. Coli bacteria which have been recultured and incubated on nutrient agar media (NA) moved on media Nutrients Broth which has containing Sterile Cryoprotectant Agent. Preservation of S. aureus and E. Coli bacterial isolates was carried out for 1- 3 weeks. Each was repeated 3 times with additions glycerol 30%, 40%, and 50%. Results test the can made-based in determine Cryoprotectant Agent best. growth research macroscopic bacteria S. aureus and E. Coli preserved on glycerol 30%, 40%, and 50% is best preserved using 30% Glycerol, while for growth microscopic S. aureus And E. Coli Which preservatif 30%, 40% and 50% glycerol, the best preservation is using 40% glycerol.

INTRODUCTION

The process and technique of storing bacterial cultures in the laboratory must be considered, because it is closely related to changes in the properties of stored bacteria. Bacterial culture storage and preservation techniques require a long and complicated time. This storage technique is related to its main purpose, which is to reduce bacterial metabolism, so that the viability of the bacteria can be maintained, and the bacterial culture remains good and there is no change in morphological properties (Rasyidah & Fariani R, 2021).

Knowledge of good bacterial culture storage techniques has the advantage of improving the quality of results, test materials, examinations, correction materials, comparison materials, and being a culture that is referred to in an effort to increase the wealth of bacterial inventory in the laboratory (Rosmania & Yuniar, 2021).

The purpose of short-term or long-term preservation is adjusted to the objectives of the program to be designed. Short-term maintenance is used for routine activities such as research or programmed projects. Long-term maintenance is related to collection and conservation efforts, if one day the microbes are needed to be available. The collection and maintenance of microbes is aimed at private or non-commercial institutions and private or commercial institutions. Making microbial collections, its success is determined by technology, available facilities, and skilled, diligent and routine labor (Shovitri, 2021).

Various kinds of studies or research conducted in the Microbiology Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Surabaya State University mostly use microbial isolates as test materials. Therefore, it is necessary to collect, store and maintain microbes properly. Important bacteria that are often used are *Escherichia coli* and *Staphylococcus aureus* (Okviandari & Sugiharto, 2019). These two bacteria require good handling and proper preservation so that their viability is

maintained. On the other hand, bacterial culture storage space (refrigerator) is limited, so it is important to conduct research on the macroscopic and microscopic growth of *S. aureus* and *E. Coli* bacteria preserved in 30%, 40%, and 50% glycerol. To find the right method of storing *Escherichia coli* and *Staphylococcus aureus* bacteria on glycerol medium.

METHODS

Sterilization of Tools and Materials

Tools and materials were sterilized first. All materials and tools were put into an autoclave and sterilized at 121^{o}C for 15 minutes with a pressure of 1 atm.

Media Preparation and Cryoprotectant Agent Preparation

Preparation of Nutrient Agar (NA) media for bacterial reculture and Nutrient Broth (NB) that has been homogenized as a medium for Cryoprotectant Agent. Preparation of Cryoprotectant Agent glycerol using the method from [2] (Rasyidah, and Fariani, 2021) which has been modified. Glycerol with a concentration of 30%, 40%, and 50% respectively was added to Nutrient Broth (NB) media. Media containing Cryoprotectant Agent glycerol 30%, 40%, and 50% were sterilized for 15 minutes with an autoclave at 121^{o}C, pressure 1 atm (Trimulyono, 2013).

Rejuvenation of Bacterial Isolates

Bacterial isolates of *S. aureus* and *E. Coli* were recultured by inoculating one full ose into Nutrient Agar (NA) media. This rejuvenation was carried out aseptically, the bacterial isolates were then incubated for ± 24 hours at a temperature of 25^{o}C-30^{o}C until colonies formed.

Isolate Preparation in Cryoprotectant Agent

Bacterial isolates of *S. aureus* and *E. Coli* that have been recultured and incubated on Nutrient Agar (NA) media are transferred as much as one full ose on Nutrient Broth media that already contains sterile Cryoprotectant Agent.

Preservation of Isolates in Refrigerator

Preservation of *S. aureus* and *E. Coli* bacterial isolates was carried out for 1-3 weeks. Each replicate was 3 times with the following conditions:

Tube 1: NB + 30% Glycerol + *S. aureus*
Tube 2: NB + 40% Glycerol + *S. aureus*
Tube 2: NB + Glycerol 50% + *S. aureus*
Tube 3: NB + Glycerol 30% + *E. coli*
Tube 4: NB + Glycerol 40% + *E. coli*
Tube 4: NB + 50% Glycerol + *E. coli*

Falcon tubes containing bacterial cultures and Cryoprotectant Agent were then stored in a refrigerator at -3°C for 1 - 3 weeks. Periodically every week a viability test was carried out (Pujiyanto, 2020).

Viability Test

Viability tests were carried out periodically by reculturing on Nutrient Agar (NA) media

in petri dishes, then incubated for ± 24 hours at 25°C-30°C until colonies formed. Microscopic observation of bacterial cultures was also carried out to ensure unchanged morphology.

Data Collection Technique

This research is experimental research. Data from the results of Preservation of Isolates in the Refrigerator is then tested for viability periodically with replication 3 times in each tube, obtained data in the form of bacterial growth and the results of macroscopic and microscopic observations. The test results can be used as a basis in determining the best Cryoprotectant Agent (Ekowati, 2020).

Data Analysis Technique

Data obtained in the form of bacterial growth and the results of macroscopic and microscopic observations. The data can be used to determine the best Cryoprotectant Agent for the test bacteria.

RESULT AND DISCUSSION

According to the length of time, bacterial culture storage techniques are divided into 3 types, namely short-term, medium-term, and long-term storage. Short-term storage is usually done by periodically rejuvenating bacteria and stored at a cold temperature not frozen (Refrigerator) (Sjarif, 2018). Medium-term storage is done by storing bacteria in mineral oil media, sterile soil, liquid paraffin, sterile distilled water, gelatin plates, and P2O2 in a vacuum (Wirasuta, 2020). Long-term storage can be done by freeze drying or other names lyophiliation and cryopreservation (Al- Humaid, 2020). Cryopreservation technique or frozen storage is a technique for storing bacterial cultures at very low temperatures (-196°C).

The test bacteria used in this study were *S.aureus* and *E.coli*. *Staphylococcus aureus* bacteria are Gram-positive bacteria that often infect humans and animals. *S. aureus* bacteria are also facultative anaerobic bacteria that can live with oxygen through aerobic respiration, but can also live without oxygen through fermentation or anaerobic respiration (Yanti, 2020). *Escherichia coli* is a negative gram bacteria with a rod shape that is aerobic or facultative anaerobic (Lolo, 2016). *S.aureus* and *E.coli* bacteria are often used as test bacteria because they are representatives of bacteria in their respective Gram, and these bacteria can adapt to universal media such as Nutrient Agar (NA) and Nutrient Broth (NB) (Unakal, 2022).

In this study, cryopreservation material in the form of glycerol was used. According to Rohadi et al., (2020) glycerol can be used as a cryoprotectant because it has a function as an osmoregulator that reduces cell dehydration during the freezing process. The addition of Glycerol to the growth medium can be used as a cryopreservation material for short to medium term bacterial cells (Guthrie, 2016).

Viability test was conducted to determine the presence and ability of bacteria after treatment for several weeks of observation. The viability test results of *E.coli* and *S.Aureus* bacteria during storage showed a decrease in growth both macroscopically and microscopically. The best cell growth in *E. coli* on microscopic observation was best treated with the addition of 40% Glycerol. In *S. aureus* bacteria, the best growth based on microscopic observation is with the addition of 30% Glycerol.

The growth rate of *E. coli* and *S. aureus* bacteria can be seen in Figure 1 below:

Tabel 1. Growth of *S. aureus* bacteria based on microscopic observation of the Viability Test

Lama Penyimpanan	0	1	2	3	
SA 30%	56.000.000	86.000.000	40.200.000	252.200.000	
SA 40%	56.000.000	49.200.000	23.600.000	29.600.000	
SA 50%	56.000.000	41.200.000	48.200.000	40.800.000	

Tabel 2. Growth of *E. coli* bacteria based on microscopic observation of the Viability Test

Lama Penyimpanan	0	1	2	3	
EC 30%	47.200.000	40.000.000	42.800.000	33.000.000	
EC 40%	47.200.000	22.600.000	32.400.000	34.000.000	
EC 50%	47.200.000	106.600.000	162.800.000	48.000.000	

Microscopic observations in each week showed the effect of the addition of cryopreservation material. For *S. aureus* bacteria, the data obtained in week 1 treatment with the addition of 30% Glycerol increased the highest number of cells, for week 2 and week 3 the addition of 30% Glycerol formed a graph with the highest cell trend, so it can be concluded that the addition of Glycerol to the best *S. aureus* culture media is at a concentration of 30% addition (Wind, 2019). In the microscopic observation of *E. coli*, the data obtained from week 1 to week 3 of the addition of 40% Glycerol can form a graph with an increasing trend in the number of bacterial cells, so it can be concluded that the addition of 40% Glycerol to *E.coli* media is the best concentration as a cryopreservation material. The addition of glycerol can minimize cell damage during the freezing process, so glycerol is one of the cryoprotectants often used by researchers (Huang, 2014).

In this study, cryopreservation material in the form of glycerol was used. According to Rohadi et al., (2020) glycerol can be used as a cryoprotectant because it has a function as an osmoregulator that reduces cell dehydration during the freezing process. The addition of Glycerol to the growth media can be used as a short to medium term cryopreservation material for bactery cells. (Romano, 2019).

CONCLUSION

The best macroscopic growth of S. aureus and E. Coli bacteria preserved in 30%, 40%, and 50% glycerol is preservation using 50% glycerol, while for microscopic growth of S. aureus and E. Coli preserved in 30%, 40%, and 50% glycerol the best is preservation using 40% glycerol.

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