IDENTIFICATION OF AIR BACTERIA USING GRAM STYING METHOD

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ABSTRACT

Microbiology is the study of microorganisms which cannot be seen with the naked eye to examine what is contained in microorganisms. To see microorganisms, it is necessary to have gram stain which is one of the most widely used procedures to characterize many bacteria. This study aims to understand the chemical and theoretical basis for the differential staining procedure as well as the procedure for differentiating between the two main groups of bacteria namely gram positive and gram negative.

The method used in this research is the experimental method. The experimental method is a method used in laboratory research. The purpose of this experimental method is to determine the consequences of a treatment given deliberately by the researcher. In addition, a qualitative descriptive approach was used to provide an overview of airborne bacteria by using the gram stain method.

The results showed that samples of NA media in airborne bacteria were carried out using a microscope at 10x magnification, which aims to see the color of the bacteria, in the gram staining experiment that has been carried out produces a purple color. This shows that these bacteria are included in the gram-positive bacteria group, as well as the results of the type of bacteria obtained in the gram stain experiment, namely *E. coli bacteria* which has aform *monobacillus*.

Keywords: Airborne bacteria, Gram stain

INTRODUCTION

Microbiology is the study of microorganisms that cannot be seen with the naked eye to examine what is contained in microorganisms. Microorganisms that exist in nature have distinctive morphology, structure and characteristics, as well as bacteria [4]. The living bacteria are almost colorless and contrast with water, where the bacterial cells are suspended.

Bacteria can be found in almost any place: in soil, water, air, in symbiosis with other organisms or as agents of parasites (pathogens), even in the human body. In general, bacteria are 0.5-5 μm in size, but there are certain bacteria that can be up to 700 μm in diameter, namely *thiomargarita*. They generally have a cell wall, like plant and fungal cells, but with very different building blocks (peptidoglycan). Some types of bacteria are motile (able to move) and this mobility is caused by flagella. One way to observe the shape of a bacterial cell so that it is easy to identify is by means of staining or staining. It also serves to determine its physiological properties, namely knowing the reaction of the bacterial cell wall through a series of stains.

The purpose of staining is to give the bacteria a color that in the end can be identified easily. In addition, there is an

endospore that can be stained. Endospores are organisms that are formed under stressful conditions due to lack of nutrition, which have the possibility to continue in the environment until conditions become favorable [6].

Gram stain is one of the most widely used procedures for characterizing many bacteria. From gram staining, cell morphology can be seen, including characteristics of gram, cell shape, and cell arrangement. Gram staining or the gram method is an empirical method for differentiating bacterial species into two major groups, gram positive and gram negative, based on the chemical and physical properties of their cell walls, this method is named after its discoverer, scientist Denmar khans Christian gram. Gram staining is divided into two, namely compound coloring because it uses more than one kind of dye. And differential staining because this staining is able to differentiate or differentiate bacteria, so that bacteria can be classified into two, namely Gram negative and Gram positive [11].

The gram staining technique must be in accordance with the procedure because it can lead to misidentification of data whether it is gram positive or gram negative so that this experiment is needed in order to know the operation of the gram staining mechanism [7].

Gram

stain Gram stain or the Gram method is an empirical method for differentiating bacterial species into two major groups, namely gram-positive and gram-negative, based on the chemical and physical properties of their cell walls. This method is named after its inventor, the Danish scientist Hans Christian Gram (1853-1938) who developed this technique in 1884 to differentiate between *pneumococcus* and bacteria *Klebsiellapneumoniae*.

The Gram stain method is also a very useful differential stain and is most widely used in microbiology laboratories, as it is an important step in the initial steps of identification. The principle of coloring is the presence of ionic bonds between the cellular components of the bacteria and the active compound of the dye called chromogen. This staining is based on the thickness or thinness of the peptidoglycan layer on the cell wall and the amount of fat layer on the bacterial cell membrane. Types of bacteria based on gram staining are divided into two, namely gram positive and gram negative [4].

Gram-negative bacteria are bacteria that do not retain the methyl purple dye in the Gram stain method. Gram positive bacteria will retain the dark purple methyl dye after washing with alcohol, while gram negative bacteria will not. In the Gram stain test, a counterstain dye is added after methyl purple, which turns all gram-negative bacteria red or pink. This test is useful for classifying these two types of bacteria based on differences in the structure of their cell walls. The differences between gram-positive and gram-negative:

1. Bacteria Gram-negative, bacteria Gram-negative bacteria are bacteria that do not retain the methyl purple dye in the Gram stain method. Gram-positive bacteria will retain their dark purple color after washing with alcohol, while gram-negative bacteria will not.
2. Gram Positive, bacteria Gram positive bacteria are bacteria that retain the methyl purple dye during the Gram stain process. These types of bacteria will stain blue or purple under a microscope, while gram-negative bacteria will appear pink. The difference in classification between the two types of bacteria is mainly based

on differences in the structure of the bacterial cell wall [1].

Bacteria have various forms of morphology, namely, round, rod and spiral.

1. Rod-shaped bacteria The rod-shaped bacteria are known as bacilli. The word basil comes from bacillus which means stem. The form of bacillus can also be divided into:
   a. Single bacillus, which is a bacterium that is only in the form of a single stem, for example salmonella typhi, which causes typhus.
   b. Diplobasil is a bacterium in the form of a rod that holds two or two.
   c. Streptobacil is a rod-shaped bacterium that extends together to form a chain, for example, Bacillus anthracis, which causes anthrax disease.
2. Ball-Spherical bacteria are known as Coccus. These bacteria can also be divided into:
   a. Shaped bacteriaMonococcus, which is a single spherical bacterium, for example Neisseria gonorrhoeae, which causes gonorrhea.
   b. Diplococcus, which is some ball-shaped bacteria that works together with two or two, for example, Diplococcus pneumonia, which causes pneumonia or pneumonia.
   c. Sarkina, which is a spherical bacterium in groups of four so that it looks like a cube.
   d. Streptococci, which are spherical bacteria that cluster lengthwise to form chains.
   e. Staphylococci, which are ball-shaped bacteria that colonize to form a group of irregular cells so that they look like a bunch of grapes.
3. There are three kinds of spiral forms:
   a. Spiral-shaped bacteria Spiral, which is a group of bacteria that looks like a spiral, such as Spirillum.
   b. Vibrio, this is considered as an imperfect spiral form, for example, Vibrio Cholera, which causes cholera.
   c. Spirochaetes, which are spiral-shaped bacteria that are flexible.

When moving, the body can lengthen and contract [6].

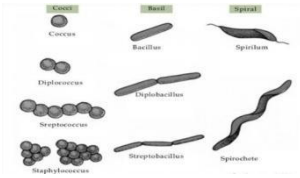


Figure 1. Bacterial Shape

Table 1. Relative Personality Differences Gram Positive Bacteria and Gram negative

Properties	Bacterial gram (+)	Bacteria Gram (-)
The composition of the cell wall	lipid content is low (1-4%)	of high lipid content
Resistance to penicillin	More sensitive	Less susceptible to
inhibition by the dye bases (VK)	More inhibited	Less inhibited
Nutritional requirements	Most species are relatively complex	Relatively simple
Resistance to physical treatment	More resistant	Less resistant

Gram-negative bacteria have 3 cell wall layers. The outermost layer, namely the lipo posaccharide (lipid), may be washed by alcohol, so that when it is stained with safranin it will turn red. Gram-positive bacteria have a thick layer of peptidoglycan cell wall. After staining with crystal violet, the pores of the cell walls are narrowed due to decolorization by alcohol so that the cell walls retain their blue color [6].

Gram positive bacterial cells may appear red if the decolorization time is too long. Meanwhile, gram-negative bacteria will appear purple if the decolorization time is too short [6].

Bacillus subtilis is a gram-positive rod-shaped bacterium, and is often found naturally in soil and vegetation. *Bacillus subtilis* grown in various mesophilic temperatures ranging from 25-35^{0}C. *Bacillus subtilis* has also evolved to be able to live even though under harsh conditions and faster to obtain protection against stress situations such as conditions of low pH (acid), alkaline, osmotic, or oxidative conditions, and heat or ethanol. These bacteria have only one DNA molecule which contains a set of chromosome sets. Its DNA size is BP 4214814 (4.2 Mbp) (TIGR CMR). 4,100 protein gene codes. Some of the advantages of this bacterium is that it is able to secrete large amounts of antibiotics out of cells [9].

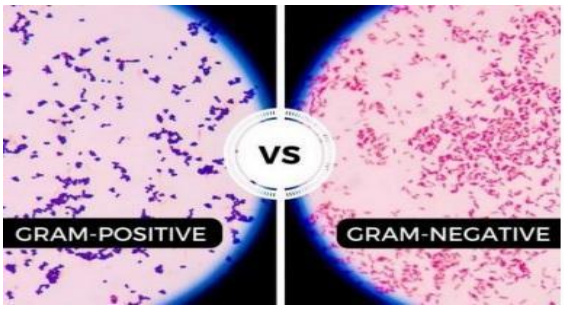


Figure 2. Bakteria Gram Negatif-Positif

Methods for gram stain:

The gram stain procedure begins with application of an alkaline dye, Crystal violet. The iodine solution is then added, all bacteria will be stained blue in this phase. The cells are then given alcohol. Gram-positive cells will still bind crystal violet-iodine compounds, remain blue, color negative cells are lost by alcohol. As a final step, countersain (eg safranin red dye) is added, so that gram-negative cells, which are colorless, will take on a contrasting color, while gram-positive cells appear in blue. The basis for the difference in the gram reaction is the structure of the cell wall [13].

Gram stain gives good results, if used fresh cultures aged 24-48 hours. When old cultures are used, there is a possibility of deviations from the results of the gram stain. In old cultures, many cells have damage to their cell walls. This damage to the cell walls causes the dye to come out when washed with a bleaching solution. This means that gram-positive bacteria with damaged walls can no longer maintain the crystal violet-iodine color complex so that they appear as gram-negative bacteria [8].

The following points to consider in gram staining are as follows:

1. The most critical phase of gram staining is the decolorization stage which results in iodine being released from cells. Do not give excessive alcohol which will cause excessive decolorization so that gram-positive cells look like gram-negative. But also don't put too little in the drop of alcohol that won't dissolve iodine completely so that gram-negative cells are like gram-positive.
2. The best gram stain preparation is to use young cultures that are not longer than 24 hours. Age of culture will affect the ability of cells to absorb the main color, especially in gram-positive. Perhaps the gram variable will show one type of cell, some colored purple and some red due to the influence of age.

Classification of Bacteria

Classification of Bacillus subtilis

Kingdom: Bacteria
Phylum: Firmicutes
Class: Bacilli
Order: Bacillales
Family: Bacillaceae
Genus: Bacillus
Species: Bacillus subtilis [9].

Bacillus subtilis is a Gram-positive rod-shaped bacterium. These bacteria are composed of peptidoglycan, which is a polymer of sugars and amino acids. Peptidoglycan which is found in bacteria known as murein. Cells form a barrier between the environment and bacterial cells which are useful for maintaining cell shape and with standing cells with high internal turgor pressure [13].

Escherichia coli belongs to the Enterobacteraceae family, which is gram-negative and fermentative rod-shaped. *E. coli* lives in large numbers in the human intestine, which helps the human digestive system and protects it from pathogenic bacteria [7]. However, the new strain of *E. Coli* is a dangerous pathogen that causes diarrheal disease and advanced diarrhea syndrome and hemolytic uremic (hus). Its beneficial role is that it can be used as an experimental waste in water, an indicator of the level of water pollution and the detection of pathogens in human feces caused by *Salmonella typhi* [19]. *Escherichia coli* is also a type of bacteria that normally lives in the digestive tract of both humans and healthy animals. The name of this bacterium is taken from the name of a bacteriologist who comes from Germani, namely Theodor von Escherich, who succeeded in isolating this bacterium for the first time in 1885. Dr. Escherich also succeeded in proving that diarrhea and gastroenteritis that occurs in infants is caused by the Escherichia coli bacteria.

Classification *E. coli*
Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Enterobacteriales
Family: Enterobacteriaceae
Genus: *Escherichia*
Species: *E. coli* [19].

In general, Escherichia coli bacteria only recognize one type of culture, namely by sexual or vegetative means. This breeding takes place quickly, if external factors are favorable to him. If external factors are favorable, then after division, the new cells will grow until each of them becomes the size of the parent cell [10].

According to literature, *E. coli* belongs to the Enterobacteraceae family, which includes gram-negative and fermentative rod-shaped bacteria. *E. Coli* lives in large numbers in the human intestine, which helps the human digestive system and protects it from pathogenic bacteria. However, the new strains of *E. coli* are dangerous pathogens that cause diarrheal disease, advanced diarrhea syndrome, vomiting, hemolyticuremic (hus), intestinal infections, urinary tract infections, and neonatal meningitis. Its beneficial role is that it can be used as an experimental waste in water, an indicator of the level of water pollution and detecting pathogens in human feces caused by *Salmonella typhi*. In addition, *E. coli is* widely used intechnology genetic engineering and is commonly used as a vector to insert genes which is desired to be developed because these bacteria have a very fast growth and are easy to handle [5].

The introduction of microbial forms (morphology), except for microalgae, must be stained so that they can be observed clearly [11]. Bacterial life is not only influenced by external factors but on the contrary, bacteria are able to influence the state of the environment, for example, it can cause fever (heat) due to being infected by the Escherichia coli bacteria in the digestive tract and causing prolonged diarrhea. If Escherichia coli is in a medium containing a carbon source (glucose, lactose, etc.) it will change the degree of acid (pH) in the medium to become acidic and will form gas as a result of the process of breaking down glucose into other compounds.

RESEARCH METHOD

Experimental

Method The experimental method is a method used in laboratory research. The purpose of this experimental method is to determine the consequences of a treatment given deliberately by the researcher. The experimental method is a research method used to look for the effect of certain treatments on others under controlled conditions [14]. Based on the definitions of several experts, it can be understood that experimental research is research conducted to determine the effect of giving a treatment or treatment on the research subject. The aim of experimental research is to examine the effect of a particular treatment on the symptoms of a particular group compared to other groups using a different treatment [20].

DESCRIPTION

Materials

1. Methylene Blue
   Official name: Methylthionini Chloridum
   Another name: Methylene blue
   RM/BM: $C_{16}H_{18}CIN_3S.3H_2O$/373.90
   Critical apparatus: Crystals or crystal-line powder dark green, bronze odor-less
   Solubility: Soluble in water and in chloroform
   Storage: In a well closed container. Use: As the main paint in simple painting [3].
2. Carbolic fuksin
   Name: Magenta Other
   Names: Karbol fuksin critical apparatus: Powder crimson
   Solubility: Water soluble
   Storage: In a tightly closed container,
   Uses: As dye bases.
3. Crystal Violet
   Official name: Crystal violet apparatus: crystals green old
   Solubility: Difficult to dissolve in water
   Storage: In a well-closed container
   Uses: As a main paint or gram A [3].

Tools

The tools used at the time of the experiment are: microscopes, bunsen burners, coloring trays, glass objects, ose/inoculation needles

Materials

Materials used at the time of the experiment, namely; air bacteria cultures, tool bacteria cultures, crystal violet, iodine grams, ethyl alcohol, and safranin.

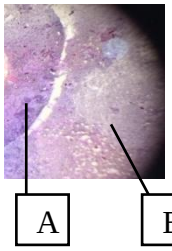
Work Procedure

1. Prepared clean,
2. Slides Prepare each smear of three organisms and to one slide. This stage is carried out by placing a drop of water on a slide, and then moving each organism separately from the water drop with a sterile loop and cooling it,
3. Allow the smear to dry in air and then heat fixation as usual. Flood the smear slowly with crystal violet and leave it for 1 minute,
4. Rinsed the smear with tap water slowly,
5. Soak the smear slowly with iodine bleach and leave it for 1 minute,
6. Rinse with tap water is slowly,
7. Bleach the color with 95% ethyl alcohol,
8. Shampoo with tap water slowly,
9. Given dye counter with safranin for 45 seconds,
10. Rinsed with water flowing slowly,
11. Dried with paper biboluous and observed under the objective lens.

RESEARCH RESULTS

Based on research conducted observational data obtained are:

Table 2. The results of gram staining observations

No	Image Bacteria	Information
1	 Media Sample NA Air Bacteria	a. Show purple bacteria (gram-positive bacteria) b. Shows the form of bacteria, namely monobacillus .

Based on the gram staining study carried out under a microscope with a magnification of 10x, which aims to see the color of the bacteria, the gram staining experiment was carried out to produce a purple color. This shows that these bacteria are included in the gram-positive bacteria group, this is because at the gram staining stage after being given a solution of Crystal violet, lugol, safranin, and washing with bacterial alcohol which looks purple, as well as the results of the type of bacteria obtained in the experiment. Gram stain was *E. coli bacteria* [21]. In the next procedure with a magnification of 100x under a microscope which aims to see the shape of the bacteria observed, the results are that these bacteria have a monobacillus shape [12].

DISCUSSION

Gram stain is a stain used to classify gram-positive and gram-negative bacteria. Gram positive bacteria will retain the crystal violet dye and will appear dark purple under a microscope. Meanwhile, gram-negative bacteria will lose their crystal violet dye after washing with alcohol, and when they are given water dye fucsin or safranin they will appear red [22].

The difference in dye is caused by differences in the chemical structure of the cell walls. Dyes used in gram stain include crystal violet, alcohol, safranin, and iodine [8]. The purpose of this Gram stain is to make it easier to see bacteria microscopically, to clarify the size and shape of the bacteria, to see the structures in bacteria such as cell walls and vacuoles, and to produce physical and chemical properties typical of bacteria with dye. In staining, Gram positive bacteria stain purple while Gram negative bacteria stain red [14].

The purpose of gram staining is to identify microorganisms, which types of bacteria and bacteria can be identified according to their categories, namely gram-positive and gram-negative, one of the bacteria has several forms, namely bacillus (rod), coccus (round), and spirilum (curved) [12]. Bacillus-shaped bacteria are divided into *diplobacillus* and *tripobacillus*. In the form of *coccus* divided into *monococcus*, *diplococcus*, to *staphylococcus* (looks like grapes). Specifically, spirilum is only divided into two, namely half curved and not curved [10].

In this study only the Gram stain method was used to identify bacteria with their advantages and disadvantages. The advantage is that Gram stain is one of the simplest and inexpensive methods for rapid diagnosis of bacterial infection [16]. This method is much faster than bacterial culture, and serves as an initial guideline for deciding on antibiotic therapy before definitive evidence is available of specific infection-causing bacteria [16].

The drawback of this method is that it can only determine the size and shape of the bacteria and see the structure in the bacteria using dyes only. The condition of Gram stain and bacterial morphology sometimes changes due to antimicrobial therapy [6]. Gram negative rod species can become filamentous and pleomorphic

whereas Gram positive bacteria can become variable after antimicrobial therapy [19].

In the first research we did was to prepare tools and materials. The tools used are petri dishes, glass objects, bunsen, ose, dropper, and microscope. While the materials used are NA, purple crystal, iodine, 70% ethanol, safranin, and aquadest.

The next step is to take bacteria from the isolation media using ose. Ose is also heated on top of the bunsen so that the condition is aseptic. After that, it is touched on a medium that has no bacteria, because if the conditions are too hot the bacteria can die. After that, bacteria are taken and scratched on the glass object. Then a glass object was fixed near the bunsen to condition the aseptic and to expand the internal and external structures of cells and microorganisms. Then drop with purple crystals using a dropper and let stand for 1 minute. This is because at 1 minute it is assumed that the purple crystal has locked and the function of the purple crystal itself is as a primary dye [22].

Note that crystal violet or purple is a triarylmethane dye. This stain is used as a histological stain in the gram method of bacterial classification. Crystal violet has antibacterial, fungal and deworming properties, and was previously important as a topical antiseptic [15].

After that, rinsed with aquadest, this is so that the remaining purple crystals that can fade and clarify the observation. After rinsing with aquadest, then dripping with iodine and left for 2 minutes, iodine functions as a purple crystal color amplifier, and is left to stand for 2 minutes because at that time it is assumed to have quite clearly given the bacteria a purple color. In contrast to the previous study, the gram staining procedure was carried out for only 1 minute after dripping with iodine and other materials, the results of this study found several types of bacteria, but the indwelling process for 1 minute in this study did not provide clear color results during identification [12].

After that, rinse again with aquadest, this aims to clean the remaining iodine in bacteria. Then washed with 70% ethanol serves to dissolve fat, then rinsed again with aquadest. This aims to clarify observations. After that drip with safranin. Safranin is a biological stain used in histology and cytology [20]. Safranin is used as a conterstain in several staining protocols. Safranin has a chemical structure [20]. In addition, Fafranin functions as a secondary dye and as a sign that the bacteria are Gram negative [18].

Allow 10 minutes because this time it is assumed that the cell wall has locked onto the safranin. Then rinsed with aquadest, this aims to rinse the remaining safranin and to clarify observations [7]. Then the final step is to observe the preparation under a microscope. After observing it under a microscope atmagnification 10x, this 10x magnification is used to see the color of the bacteria, in the gram staining research that has been carried out it produces a purple color [17].

This shows that these bacteria are included in the gram-positive group of bacteria, as well as the results of the type of bacteria obtained in the gram stain experiment, namely *E. coli bacteria*. In the next procedure with a 100x magnification under a microscope which aims to see the shape of the observed bacteria, the result is that the bacteria have ashape *monobacillus* [7].

Escherichia coli is a normal bacterial flora in the human intestine, spread in the environment through water or equipment contaminated with human feces [4]. Water is the only vehicle for transmission of oral faecal pathogens such as

Escherichia coli. Contaminated water, hand held hands, laboratory equipment, and laboratory clothing are risk factors that play a role in increasing the risk of bacterial contamination [19]. These bacteria will turn into pathogens and cause infection if they are outside their normal habitat (outside the intestine) such as the skin [4]. This contamination can occur directly from the laboratory environment or laboratory personnel [4].

Gram-positive bacteria have a simpler cell wall, with relatively large amounts of peptidoglycan [5]. Meanwhile, the cell wall of gram-negative bacteria has relatively little peptidoglycan and is structurally more complex. The outer membrane of the gram-negative cell wall contains lipopolysaccharides, which are carbohydrates that are bound to lipids [5]. Among the pathogenic bacteria that cause disease, gram-negative species are generally more dangerous than gram-positive species [6].

The response to the inhibition of gram-positive microbes was stronger than that of gram-negative microbes. This is due to differences in the components of the cell wall between gram-positive and gram-negative microbes. The cell wall of gram-positive microbes contains a lot of theikoronate and polysaccharide molecules [7]. These chemical components protect cells from enzyme lysis, while other substances determine the cell's reaction to gram staining and some attract and bind bacteria [7].

The results of this study are in line with previous research which stated that in his research there were bacteria genus/species of bacteria in thegroup *Micrococcus sp*on NA media of air bacteria samples in class [10]. Judging from the results of these studies that in identifying gram-positive and gram-negative bacteria using gram staining, the results are faster and more effective. As for previous studies that used the gram staining method to identify gram-positive and gram-negative bacteria, the results of this study were that several types of bacteria were found in the pavilion inpatient room [2]. The bacterial variations are *Staphylococcus sp* and *Staphylococcus epidermis* [9].

The bacteria found are airborne contaminants [15]. The contamination is usually transmitted through the body/hands, the equipment used in the laboratory, and the clothes of the personnel used in the laboratory [15]. Therefore, it is very necessary to carry out periodic and regular checks for air sterilization, the environment of the laboratory room, the clothes of the staff and the tools used in the laboratory [14]. This can help minimize germs in the laboratory [14].

CONCLUSION

From the results of the study it can be concluded that in the airborne bacteria NA medium there are bacillus sp bacteria which are included in the gram-positive bacteria group which has a monobacillus form. The bacteria identified in the laboratory room air may be a type of contaminated bacteria from the body/hands and research clothes as well as some tools used in laboratory operations.

The hope for future researchers is related to air bacteria using the gram staining method so that they can use the tools and materials to be used in the experiment in a sterile state.

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