

MAKING NA AND PDA MEDIUM AS MICROORGANISM GROWTH MEDIA

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ABSTRACT

Microorganisms can be grown and developed on a substrate called medium. With the medium of growth, microbia activity can be studied and with the growing medium can be done isolation of microbes with pure culture, propagation, testing of physiological properties, and calculation of the number of microbes.

This study aims to find out how to make growth media NA bacteria and PDAs. The method used in this study is an experimental method conducted at the Microbiology Labolatorium of Bina Mandiri University gorontalo.

The results showed that the creation of na and PDA bacterial growth media was successfully carried out. Medium function (NA) based on the chemical composition is a non-synthetic or semi-scientific medium, based on consistently a solid medium, to grow bacteria e.g. in meat, (PDA) including solid media, based on the chemical arrangement including synthetic to grow fungi for example in potatoes.

Keywords: micro-organisms, NA and PDA

INTRODUCTION

Nowadays, with the development of science, the higher one's curiosity about what is in nature to microorganisms that can not be seen with the naked eye. From this comes the science that learns about microorganisms called microbiology. In the field of research of microorganisms, of course using special techniques or ways to study it and to work on a laboratory scale to research these microorganisms both their properties and characteristics, and it is also necessary to introduce microbiology laboratory tools and techniques or ways of using tools related to the research.

In nature there are many bacteria where the bacteria have the ability to develop and multiply themselves. In addition to nature, bacteria can also be bred by using artificial media to keep alive as a single colony. Bacteria that act as a

single colony can be used more than bacteria found in nature.

We know that all living things need nutrients for their growth and reproduction. Nutrients are raw materials used to build new cellular components and to produce the energy needed in the process of cell life. To need and breed microbes is required a substrate called medium. While the medium itself before use must be in a sterile state means not overgrown by other microbes that are not expected so that microbes can grow and breed well in the medium, then it is necessary certain conditions that include that in the medium must contain all nutrients needed for the growth and development of microbes then the composition of food, osmosis pressure , chastening, acidity (ph), temperature, sterilization. We need to know that the manufacture of media is based on its function, composition, and consistency so

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that in culture or media created can grow microbes well and in accordance with well and as expected.

Bacterial breeding is done by implanting it in a particular medium. The media can be either natural, semi-natural (semi-synthetic) or synthetic depending on the characteristics of the bacteria. In general, bacteria can grow and develop in a medium that has enough nutrients to suit their needs with a pH range between 5-6. Each medium that has been created can not always grow bacteria even though the media contains enough nutrients and has a pH between 5-6 because each bacteria needs different nutrients. In addition, there are also bacteria that cannot be grown in artificial media.

The bacterial population in our surrounding nature is very large and complex. To study these bacteria, we must be able to grow or develop them. Media is needed to grow and develop a microorganism.

Bacterial growth in addition to requiring nutrients, also requires proper pH. Most bacteria cannot grow in overly alkaline conditions. The breeding of bacteria and fungi is intended to facilitate the examination to be conducted in the laboratory, so that if at any time we need bacteria and fungi for an experiment, then the bacteria and fungi are available. Breed the bacteria and fungi can be stored in the refrigerator for a long time without any damage.

Microorganisms can be grown and developed on a substrate called medium. With the medium of growth, microbia activity can be studied and with the growing medium can be done isolation of microbes with pure culture, propagation, testing of physiological properties, and calculation of the number of microbes. [13]. Medium is an ingredient consisting of a mixture of nutrients or nutrients (nutrients) used to grow microorganisms above or in them. For this purpose, a

medium is needed as a place to grow and isolate microorganisms. Microbial breeding in the laboratory requires a medium containing nutrients as well as a growth environment that corresponds to microorganisms [14].

The best food base for bacterial growth is a medium containing organic substances such as meat stews, vegetables, food wastes or potions made by humans. [7]. Bacteria in the medium also need food for their growth. Microorganisms can multiply naturally or with human intervention. Microorganisms developed by humans among others through the growth of using media. In the manufacture of this medium, it must be understood the types of nutrients required by bacteria and also the state of the physical environment that can provide optimum conditions for its growth. Therefore, this experiment was conducted to find out how to make microbial growth medium.

Living things on earth consist not only of living things that can be seen by the naked eye, but there are also microorganisms that are small in size and can only be seen using special techniques and equipment. Microorganisms (bodies of rhymes) are living bodies that have a very small size.

Microorganisms as living beings are the same as other living organisms, in desperate need of energy and materials to build their growth, as in the synthesis of protoplasts and other parts of cells. These ingredients are called nutrients. To make use of these materials, the cell requires a number of activities, causing chemical changes in the cells. All directed reactions that take place inside these cells are called metabolism. Metabolism, which involves a variety of reactions in the cell, can only take place with the help of an organic compound called an organic catalyst or commonly called an enzyme biocatalyst. To be able to understand about these

nutrients and metabolisms, basic knowledge of biochemistry is needed.

Microorganisms affect human life either directly or indirectly that can act as friends or opponents for human life. Microorganisms can multiply naturally or with human intervention. Microorganisms developed by humans include through the growth of using media.

Fault factor in heating NA and PDA broth, the fault factor when heating na broth and PDA on the hot plate should always be considered so that the broth is not spread and pay attention when pouring the broth (NA and PDA) so as not to come out of the container that has been provided. Mix the ingredients, dissolved in water with each other and heated in a hot plate so that the solution is homogeneous. Filter with filter paper for media so that filtering should be done in hot media. Define and set the pH. pH is a factor that really affects a success in the manufacture of media so that pH conditions that are too alkaline or too acidic are not suitable to be used as microbial mediums because microbes can not live in such conditions put media into the Erlenmeyer by using paper and closed serves so that at the time of diautoclave media is not alkaline [6].

Types of media

1. Media Difrensial, it is a medium that supports the lives of some bacteria and can also distinguish different groups of bacteria. In addition to bloodused various pH indicators (pH indicators) as differentiators, for example: blue bromtimol, red phenol, neutral red.
2. Selective media, this medium inhibits the growth of certain bacteria and also allows the growth of certain bacteria. This medium is used to isolate certain bacteria. As an inhibitory material used, Violet crystals, eosin v.bin methylene and beriliant green will inhibit gram (+) bacteria.

3. Selective and difrensial media, this medium is selective and difrensial usually used for identification.
4. Media for Anaerobic Bacteria, a number of chemicals can be added to the media to reduce oxygen content by chemically binding. These ingredients are: Na thioglikolat, cystcine, ascobic acid.
5. Disseminating media, this medium will accelerate the growth of certain organisms the way it is used when one particular organism is desired in a mixed breed of bacteria. In general, this medium uses materials/nutrients similar to habitat, isolating the bacteria. Nutrients added to the medium nitrogen, carbon, vitamins and growth factors mineral water salts.

The rest of the bacteria are resistant to change. Small pH in about pH (until) rapid change did result in a change in the intracellular pH value resulting in a short lasting but within 10 minutes the previous pH value had been restored [2]. Microorganisms as living beings are the same as other living organisms, in desperate need of energy and materials to build their growth, as in the synthesis of protoplasm and other parts of cells. These ingredients are called nutrients. To make use of these materials, the cell requires a number of activities, causing chemical changes in the cells. All directed reactions that take place inside these cells are called metabolism. Metabolism, which involves a variety of reactions in the cell, can only take place with the help of an organic compound called an organic catalyst or commonly called an enzyme biocatalyst. To be able to understand about these nutrients and metabolisms, basic knowledge of biochemistry is needed [5].

Media Definitions

Medium is an ingredient consisting of a mixture of foodstuffs or nutrients to grow a microorganism. Media serves to grow microbes, isolate, multiply the

number, test the physiological properties and calculation of the number of microbes, where in the process of manufacture must be sterilized and apply aseptic methods to avoid contamination of the media. Mikroorganism that we want to grow, the first thing to do is to understand the basic needs then formulate a medium or material to be used. Water is essential for single-celled organisms as the main component of their protoplasm as well as for the entry of nutrients into cells. Medium making should use distilled water. Sadah water generally contains high calcium and magnesium ions. In mediums containing pepton and meat extract, water with sadah water quality can already cause the formation of phosphate deposits and magnesium phosphate [10].

Medium is a substance consisting of a mixture of food substances (nutrients) used for the maintenance and growth of microorganisms. Microorganisms are also living things, to maintain it is needed a medium that must contain all the substances needed for its growth, namely organic compounds consisting of proteins, carbohydrates, fats, minerals and vitamins. Medium is used to see the movement of a microoranm whether motile or nonmotile, this medium is added 50% compactor material [9].

The main role of nutrients is as an energy source, a cell building material and as an electron acceptor in bioenergetic reactions (reactions that produce energy). Therefore the necessary foodstuffs consist of water, energy sources, carbon sources, electron acceptor sources, mineral sources, growth factors and nitrogen. The jam, a common nutrient in the seeding medium should contain all the elements essential for the biological synthesis of new organisms [3].

The medium used to grow and breed microorganisms must be in accordance with the needs of the types of microorganisms concerned. Some

microorganisms can live well on very simple mediums that contain only anarchic salts in added organic carbon sources such as sugar. While other microorganisms require a very complex medium that is in the form of a medium added blood or other complex materials. [15]

Formulating a medium or material that will be used to grow microorganisms in it should pay attention to various provisions such as if we want to make a medium for single-celled organisms, usually water is very important as the main component of its protoplasm and for the entry of nutrients into cells. Making medium to be solid, used gelatin, gelatin or silica gel in order to be an ideal growing medium introduced through bacteriaological methods. [1]

Sterilization

Sterilization is a process to kill all organisms that can be found in an object. Sterilization process can be distinguished into 3 kinds, namely the use of heat (massage and hot air); filtering; use of chemicals (ethylene oxide, peracetic acid, formaldehyde and alkaline glutaraldehyde) [12].

The basic principle of sterilization is to extend the shelf life of foodstuffs by killing microorganisms in them. These decaying microorganisms can be bacteria, khamir (yeast) and mold (fungi). Physical sterilization can be done by heating & spawning:

1. Spawning (with direct fire): burning the tool on the fire directly, for example tools: inoculum needles, tweezers, L rods and others;
2. Dry heat sterilization: sterilization with oven generally at a temperature of 160-1700C for 1-2 hours. Dry heat sterilization is suitable for sterilization of unstable powders against moisture, a tool made of glass;
3. Heat vapor sterilization: this concept is similar to steaming, using hot steam under pressure using autoclaves.

Chemical sterilization, usually chemical sterilization using disinfectant compounds such as alcohol. The sterilization process of this chemical antiseptic is usually done by directly delivering to the tool or media to be sterilized. Mechanical sterilization (filtration) using a very small porous sieve (0.22 microns or 0.45 microns) so that microbes are stuck on the sieve. This process is intended for sterilization of heat-sensitive materials, e.g. serum solutions, enzymes, germ toxins, cell extracts and others. [8]

Kinds of Bacteria

Bacteria are single-celled organism that reproduces in a simple way, by means of binary cleavage. Most live freely and contain genetic information and have biosententic systems and energy generators that are essential for their growth and reproduction. And there are also a number of bacteria there that are parasitic.

1. Based on its shape, bacteria are divided into 3 large groups, 3/4 Kokus (coscus) is a bacterium that is round like a ball and has several variations, namely:
 - a. Micrococcus, if small and single,
 - b. *Diplococcus*, if it's two-two.,
 - c. *Tetracoccus*, if four-sided and square,
 - d. *Sarcina*, if it forms a cube,
 - e. *Staphylococcus*, if it's clustered,
 - f. *Streptococcus*, if it forms a chain.

Then, 3/4 Basil (Bacillus) is a group of bacteria in the form of rods or cylinders, and has variations that are:

- a. *Diplobacillus*, if it goes two-two,
- b. *Streptobacillus*, if it goes hand in hand with the chain.

Then, 3/4 Spiral (Spirillum) is bacteria that is curved and has variations namely:

- a. *Vibrio*, (comma shape), if the curve is less than half a circle (comma shape),
- b. *Spiral*, if the curve is more than half a circle,

- c. *Spirochete*, if curved form a flexible structure.

The body shape of these bacteria is usually influenced by the state of the environment.

2. Based on the place and number of flagelles owned by bacteria can be divided as follows:

- a. 3/4 Atrik, has no flagel,
- b. 3/4 Monotric, has only one flagel at one end,
- c. 3/4 Lofotrik, has a number of flagells at one end,
- d. 3/4 Amphitritris, has a flagel at both ends,
- e. 3/4 Peritrik, has flagellation on the entire surface of his body.

3. Based on how to get food, namely:

- a. 3/4 Heterotrophic bacteria, namely obtaining food in the form of organic substances from the environment (residual organisms, garbage or substances in the body organism other). Such as: *Escherichia coli*, *clostridium tetani*,
- b. 3/4 Autotrophic bacteria, which are bacteria that can compose their own organic substances from inorganic substances. Such as: *Bacterochlorophils* and *Bacteripurpurin*.

4. Based on the oxygen needs can be divided, namely:

- a. 3/4 Aerob is a bacterium that requires oxygen-free for respiratory reactions. Like: *Nitrosomonas*. Sp, *Mycobacterium tuberculosis*.
- b. 3/4 Anaerobic bacteria that do not require free oxygen for respiratory reactions, such as: *Lactobacillus bulgaricus*, *Clostridium tetani*, *Mycrococcus denitrificans*.

5. Based on the layer of cell wall peptidoglican:

- a. Gram-positive bacteria are purple bacteria, and thick cell wall peptidoglikan layer, such as:

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- Neisseria gonorrhoeae, Terponema pollidum, vibrio cholera, Bacillus sp,
- b. Gram-negative bacteria are easy red bacteria, and a thin layer of cell wall peptidoglikan, such as: Propioni bacterium, Sterptococcus metans, Staphylococcus aureus.

RESEARCH METHOD

This research is a study that uses experimental methods conducted in the microbiology laboratory of Bina Mandiri University Gorontalo. This research was conducted to create a growth medium for NA and PDA microorganisms.

Tools and Materials

Tools used in this practicum are stirrers, erlenmeyer, measuring cups, hot plates, spatulas, magnetic stirrers, and aluminum foil. The materials used in this practicum include PDA, NA, Chlorphenikol and akuades.

Working Procedure

1. Media manufacture using NA
 - a. Weighed NA weighing 1.2 grams;
 - b. Inserted NA that has been weighed into 60 ml of aqueous;
 - c. Heating na media that has been mixed with aquadest for 15 minutes, at a temperature of 121 ° C while stirring;
 - d. After the NA media emit bubbles then heating in the NA media is moved from the heater at 264 ° C;
 - e. Covered with cotton and aluminum foil and then sterilized;
 - f. Then put into the autoclave 121 ° C for 15 minutes;
 - g. After that take a petri dish that has been sterilized and pour the media that has been input into the autoclaved and let stand until it becomes solid.
2. Media manufacture using PDA
 - a. Weighed PDA weighing 2.34 grams;

- b. Inserted PDA that has been weighed into 60 ml of aqueous;
- c. Heating PDA media that has been mixed with aquadest for 15 minutes, at a temperature of 121 ° C while stirring;
- d. After the PDA media emits bubbles then heating on the PDA media is transferred from the heater at 264 ° C;
- e. Covered with cotton and aluminum foil and then sterilized;
- f. Then put in autoclaved 121 ° C for 15 minutes;
- g. After that take a petri dish that has been sterilized and pour the media that has been input into the autoclaved and let stand until it becomes solid.

RESEARCH RESULTS



Figure 1. Na and PDA Media Creation Process (in heating Process)

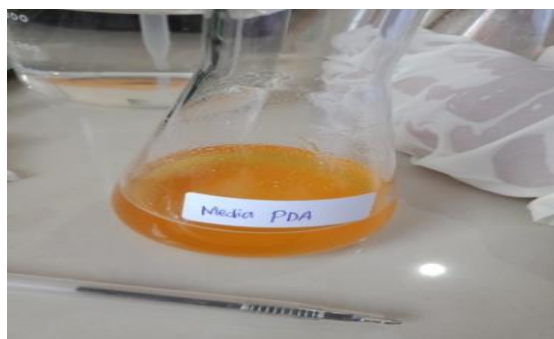


Figure 2. Na and PDA Media Creation After Process (finished in heating process)

Table 1. Research Result

No	Media	Criterion
1.	NA	Light yellow color, naivety as a place of growing bacteria.
2.	PDA	Dark yellow color, used as a place to grow mushrooms.

Source: Microbiology Practicum Report.

DISCUSSION

The discussion of the results of observations made is the medium of growth of microorganisms is an ingredient consisting of a mixture of food substances (nutrients) needed microorganisms for their growth. Media bacterial growth consists of two forms namely liquid form and solid form. Different bacteria require the need for different nutrients, so a variety of growth media are developed for use in microbiological diagnostics.

Nutrient Agar (NA)

In the manufacture of microorganism growth media using NA is required 1.2 grams. Then dissolved in 60 ml aquades. It is then sterilized in autoclaves at 121°C for 15 minutes. Once sterile, it is stored in a clean and safe place.

Nutrient order is a common medium for water testing and dairy products. NA is also used for the growth of the majority of microorganisms that are not selective, in the sense of heterotrophic microorganisms. This medium is a simple medium made from beef extract, pepton, and agar. NA is one of the commonly used media in bacteriological procedures such as regular tests of water, sewage, food products. Nutrient agar (NA) is a solid medium, which is a combination of natural ingredients and chemical compounds. In na medium, the main nutrient is meat broth. Other ingredients are pepton, bacto agar and aquades. Pepton and meat as animal proteins, vitamins, carbohydrates for bacteria.

Potato Dextrose Agar (PDA)

PDA's are used to grow or identify yeast and maca oxide. Can also be used for enumeration of yeast and anytime in a sample or food product. PDA contains a

sufficient source of carbohydrates consisting of 20% potato extract and 2% glucose so it is good for the growth of macaquis and khamir but less good for bacterial growth.

In the manufacture of growth media microorganisms using PDA required 2.34 grams. Then dissolved in 60 ml of aquades. It is then sterilized in autoclaves at 121°C for 15 minutes. Once sterile, it is stored in a clean and safe place.

The purpose of medium sterilization is so that there are no microbes and no physical changes such as discoloration, odorless, invisible surface of the medium that is not overgrown by microbial colonies (no microbial contamination occurs).

CONCLUSION

From the practicum on microbial growth media can be concluded that the growth medium of microorganisms is an ingredient consisting of a mixture of food substances (nutrients) that microorganisms need for their growth.

Medium function (NA) based on the chemical composition is a non-synthetic or semi-scientific medium, based on consistently a solid medium, to grow bacteria e.g. in meat, (PDA) including solid media, based on the chemical arrangement including synthetic to grow fungi for example in potatoes.

Advice

The advice of this practicum should be done correctly because this practicum relates to each other with the next practicum.

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