



## Microbial Isolation

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### Abstract

*The importance of isolating a microbe from the environment, such as food (solid substrate), drinks (liquid substrate), and yourself because of the many microbes that are difficult to observe or distinguish directly using the five senses. A sample can contain bacteria or fungi. By isolating, the shape of the colonies and the contents in a sample can be observed. Bacteria from the air and normal flora form colonies with lobate-shaped edges, whereas bacteria found in well water samples form colonies with irregular edges and there are also fungi found in the well water samples.*

## Introduction

Specific skills are needed in learning microbiology so that learning objectives can be achieved. In addition to learning in the classroom, practicum activities in the laboratory also need to be done because the object of learning microbiology is very difficult to observe directly in daily life. Related to this, observations need to be made in the laboratory. Observation of microorganisms can only be done if the microorganisms to be observed are isolated in certain places so that they are easily observed under a microscope.

Microorganisms live freely in the environment, spread in the air, soil, water, food, even those of microorganisms that live in the human body. Observations on certain microorganisms can only be made if microorganisms are separated from the environment and other microorganisms. This can be done with isolation techniques. Several methods are often used in microbial isolation. The methods used are also adapted to the type of microbes to be observed. Observation activities in microbiological practice cannot be separated from microbial isolation, so this practicum needs to be done.

Microbial isolation technique is an attempt to grow microorganisms outside of their natural environment. Separation of microorganisms outside the environment aims to obtain bacterial cultures that are no longer mixed with other bacteria called pure cultures. The principle of microbial isolation is to separate one type of microbe with other microbes derived from a mixture of various microbes. This can be done by growing it in solid media, microbial cells will form a colony of cells that remain in place (Lestari, 2017).

Providing samples as a source of microbial isolates can be obtained in a variety of ways there are samples obtained from soil, livestock manure, livestock rumen fluid. From the plant part. There are samples from food products such as milk, meat, fish, shrimp paste and others. There is a sampling by doing the fermentation process to obtain indigenous microorganisms (MOI) which aim to spur the process of decomposition by microbes. Microbes that have grown and developed from MOI media are used as isolate sources for isolation purposes.

Some factors that need to be considered in carrying out microbial isolation include; (1) The nature of each type of microbe to be isolated; (2) Place of life or origin of the microbe; (3) the appropriate growth medium; (4) How to inoculate microbes; (5) How to incubate microbes; (6) How to test that the isolated microbes have been in the form of pure culture and in accordance with what is intended; (7) How to maintain that the microbes that have been isolated remain pure culture.

A sample of a suspension in the form of a mixture of various species is diluted in a separate tube. Dilution of microbial suspense is generally carried out by a series of dilution techniques. From the results of this dilution then taken about 1 mL to be further diluted. If the third dilution is taken 0.1 mL to be spread on a solid medium, it is likely that we will get several colonies that will grow in that medium, but maybe we will only get one colony only. The principle of the method of isolation on the agar cup is to thin the microorganisms to obtain individual species that can be separated from other organisms. Each separate colony that appears in the cup after incubation comes from a single cell (Yunilas, 2017).

The importance of isolating a microbe from the environment, such as food (solid substrate), drinks (liquid substrate), and yourself because of the many microbes that are difficult to observe or distinguish directly using the five senses. So that the isolation will make it easier to see and observe forms of microbial growth in several mediums and can see the morphology of the microbes, namely inoculation which is a technique of transferring a certain culture from the old medium to a new medium with the aim of obtaining pure culture without contamination from microbes undesirable. Several methods are known for or methods for obtaining pure culture from a mixed culture. The two most commonly used are the scratch cup method and the pour cup method. Which is based on the principle of dilution with a view to obtaining individual species. Assuming that each colony can be separated from a type of cell that can be observed. Pure culture is needed in various microbiological methods, including being used to identify microbes. To observe the cultural characteristics of morphology, physiology, and serology, microbes from one species are needed (Harti, 2015).

Dilution of 1 g soil sample was weighed and then put into a test tube. Bacterial isolates that can be purified to obtain pure culture because colonies that grow in petri dishes still have several colonies. Peat soil purification is carried out by means of a scratch to use continuous stroke techniques. Ose needles that have been flattened to red are then cooled and then used to take bacterial colonies in a petri dish. The ose needle that has been used to take a bacterial colony is etched half in the agar plate, then the plate is rotated 180° then re-etched on the surface of the remaining agar plate (Irfan, 2014).

Isolation is done by stratified dilution then followed by the spread plate method which is carried out aseptically in Laminar Air Flow (LAF). From dilution of  $10^{-1}$  to  $10^{-7}$ , 100  $\mu$ l of the sample solution is taken using a micropipette and transferred to the surface of a solid Nutrient Agar (NA) medium. Then the inoculum was held with drigalski and incubated in an incubator at 37°C for 48 hours. Isolate characteristics include microscopic observations (characterization of shape, edges, elevation and color of colonies, and microscopic observations include cell morphology and staining (Hefdiyah, 2014).

The presence of bacterial growth in the medium shows that bacteria are able to show that bacteria are able to utilize the nutrients present in the medium (Hidayah & Shovitri, 2012), the need for bacteria in the form of energy sources that can be derived from light (phototrophs) and organic carbon (kemoorganotrof), carbon sources in the form of inorganic carbon (like potassium nitrate) and organic nitrogen (in the form of proteins and amino acids), non-metallic elements such as sulfur and phosphorus, metal elements (such as potassium, sodium, magnesium, iron, copper, etc.), water for metabolic functions and growth (Hidayah, 2012).

Some potential bacterial species of mineralization and solubilization respectively for organic and inorganic phosphorus (Sutanto, 2002). The solvent phosphorus activity is determined by the ability of microbes to release metabolites such as organic acids, through which they hydroxyl and carboxyl group the cations bound to the phosphate which are last converted to a soluble form. Several kinds of microbial solubilization mechanisms exist in nature and much of the global cycling of organic and inorganic soils that are insoluble in phosphate is linked to bacteria and fungi. Bacterial isolates are further characterized by a series of biochemical reactions (Karpagam, 2014).

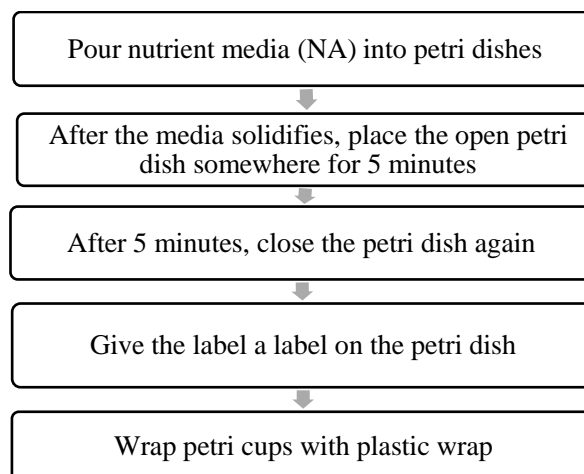
## Methods

This practicum activity is carried out with the aim of identifying the morphology of Smicroorganism colonies found in the surrounding environment and the human body. This practicum was held on Thursday, January 18, 2018, at the Microbiology Laboratory, Makassar State University

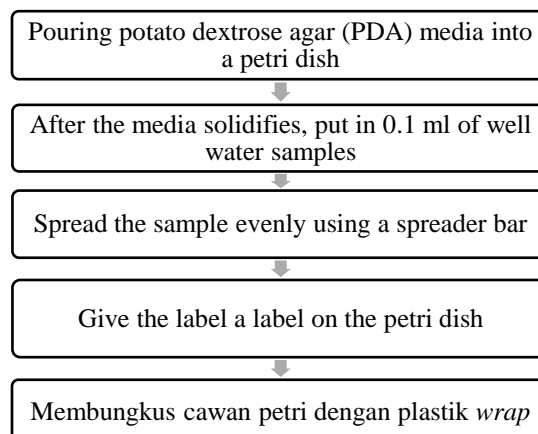
### *Tools and Materials*

The equipment used in this practicum activity is Enkas, Bunsen Burners, Petri dishes, Spoilers, ose needles, Spreader bars, Stopwatch, Cotton buds, Labels, Plastic wrap, Incubators. In this practicum used materials such as Medium nutrient agar (NA), Medium potato dextrosa agar (PDA), Well water, normal flora

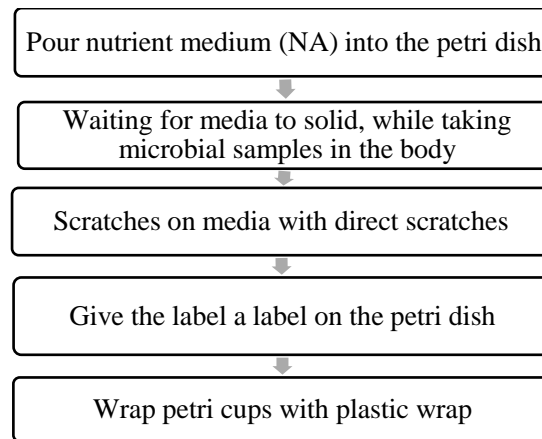
### *Capture Method*



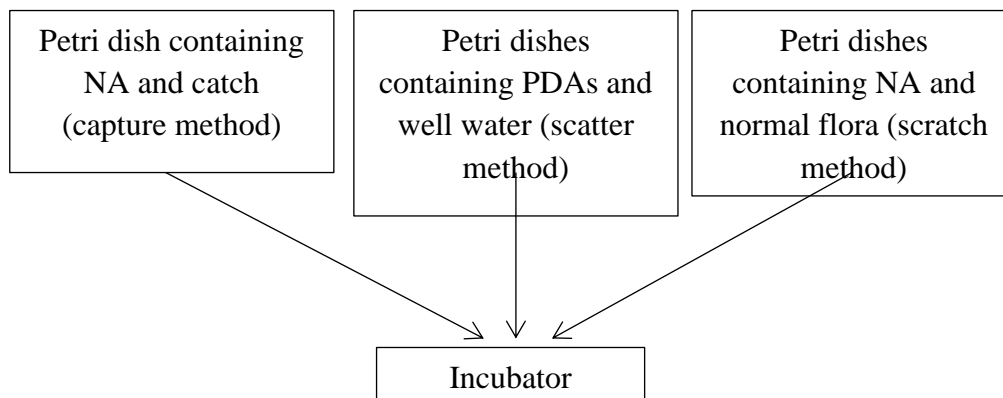
### *Scatter Method*



## Scratch Method



## Incubation and Observation



## Results and Discussion

### Observation result

Based on the analysis and observations on the practicum, the following results are obtained:

**Tabel 4.1 Microbial Isolation**

No.	Medium + Ingredients	Edge	Form	Colour	Information
1.	NA Catch	Lobate	Lobate	White	Bacteria
2.	PDA Water wells	Irregular	Fimbrante	White	Bacteria
3.	NA Normal flora (behind the ear)	Lobate	Lobate	White	Bacteria

In this practicum, three Petri dishes are used, with the first petri dish using agar medium (NA). Material or samples in the first petri dish are obtained using the catch method. After the medium is poured into a petri dish, the medium is left to solidify. After the medium is solid, the petri dish is then left open and placed in the yard of the laboratory building for five minutes. After that, the petri dish is then wrapped in plastic wrap to prevent contaminants then put in an incubator to go through the incubation stage for four days.

The second petri dish is filled with a medium in the form of potato dextrose agar (PDA). A medium of 20 mL was poured into a petri dish and then waited until it solidified. Samples in the form of well water are then put as much as 0.1 mL and spread using a spreader rod on the surface of the medium. The method used is the scatter method, where the medium is inserted first then a 0.1 mL sample is spread over it. After the sample is distributed, the petri dish is wrapped in plastic wrap to prevent contaminants. Next, the petri dish is put into the incubator to go through the incubation stage for four days.

Isolation in the third petri dish using the medium in the form of nutrient agar (NA) and using the scratch method. After the medium solidifies, microbial samples are taken from one part of the body, namely at the back of the ear. Samples are normal flora, namely microbes that are attached and live in the body in normal conditions. Samples were taken using a sterile cotton bud, then scratched with a scratch directly on the medium. The petri dish is then wrapped in plastic wrap to prevent contaminants. Next, the petri dish is put into the incubator to go through the incubation stage for four days.

After the incubation stage, observations were made on the isolated microbes. Each petri dish is opened and the morphology of the colony is formed. In the first cup, several bacterial colonies are formed. This can be seen from the white pattern on the medium. Using the instructions of the shape of the colony on the agar plate, it can be seen that the colony formed from the catch is lobate. These microbes are identified as bacteria.

Isolation in the second and third plates also shows the presence of several microbial colonies derived from samples of well water and normal flora that live in the back of one ear's practitioner. The entire colony looks white by forming certain formations. In the second cup, the shape of the edge of the colony is irregular (irregular) and can be seen the presence of mold that grows. This can be caused by the presence of mold spores in the well water used as a sample. As for the third cup, also formed several bacterial colonies that can be seen white. The shape of the edge of the colony is no different from that of the first cup, which is lobate. This may be due to the similarity of the use of the medium and the type of microbes that grow in it.

## Conclusion

Based on observations, it can be concluded that microorganisms exist in various places and live around humans and even those that live in the body. A sample can contain bacteria or fungi. By isolating, the shape of the colonies and the contents in a sample can be observed. Bacteria from the air and normal flora form colonies with lobate-shaped edges, whereas bacteria found in well water samples form colonies with irregular edges and there are also fungi found in the well water samples.

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