The Effect of Environmental Factors on Microbial Growth

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Abstract

Chemical factors that have inhibition based on the results of this practicum are Tetracycline and formalin. In this experiment, no type of liquid bath soap was found that inhibited the growth of E. coli. This can occur because of an error in the experimental procedure. Escherichia coli bacteria can grow optimally in the temperature range of 30 °C. Formalin has a strong inhibitory effect on the growth of Escherichia coli bacteria, as well as Tetracycline antibiotics which can inhibit the growth of E. coli bacteria.

Introduction

There is a perception that the number of microorganisms that live around humans and other living things is very much. Because of its very small size, it is possible to have a large number of microorganisms that live in small environments. The number of microorganisms found in a sample or material varies greatly according to the type of material and the environmental conditions of the material or sample. Microorganism growth is influenced by several factors. The main factor influencing microbial growth is the environmental factors in which it lives.

Wickramasinghe (2015) suggests that panspermia, an ancient idea, holds that microbial life is everywhere in the Universe. Essentially, the microbial environment consists of light, temperature, pH (power of hydrogen), humidity, and other chemicals. Humans sometimes make certain efforts to kill microorganisms. For example in the autoclave sterilization process that utilizes high temperatures and pressures to kill microbes in the tools and materials to be used. Certain chemicals are sometimes also used to inhibit microbial growth. For example, the use of antibiotics and soap. Chemical solutions such as formalin, alcohol, and iodine are also used to kill microorganisms and inhibit their growth so that sometimes the solution is used to prevent infection in wounds and preservation of certain tissues or materials. Activities in microbiology laboratories can investigate the effect of environmental factors such as temperature and chemicals on microbial growth.

One of the microbes that live freely in the environment and can harm humans is Escherichia coli. These bacteria can be obtained by isolation so that growth can be observed. In addition to analyzing environmental factors that affect microbial growth, by doing this practicum, it is also known what efforts should be done to kill harmful microbes.
Bacterial life is not only influenced by environmental factors but also affects the environment. Bacteria can also change the pH of the medium in which it lives, this change is called a chemical change. The environmental factors can be divided into biotic and abiotic. Biotic factors consist of living things, while abiotic factors consist of natural (physical) and chemical factors (Murwani, 2015).

According to Attoriq & Sodik (2018), various physical and chemical methods can be used to control microbes. Microbial activity is generally very dependent and influenced by environmental conditions, among others: (1) Physical factors, for example; temperature, pH, osmotic pressure, oxygen content and others; (2) Chemical factors, eg metals - toxic metals, toxic substances; (3) Biological factors, for example; antibiotics, interactions with other microbes.

According to Amaliyah (2017), the factors that influence microbial growth include; (1) Effect of Temperature. Microorganisms that have optimum temperatures between 0º -20ºC are called psychrophiles. Microorganisms that grow rapidly in the temperature range of 20º - 50º C are called mesophiles, while microorganisms that grow in the temperature range of 50º - 100º C are called thermophiles. Some microorganisms can survive at high temperatures even though these temperatures cannot grow, this group is called thermoduric. Spore-forming microorganisms can survive boiling temperatures for 5-15 minutes because they are heat-resistant; (2) Effect of pH. Microorganisms that carry out the fermentation process produce acid so that the pH can drop to 3.5. Conversely, when the metabolism of proteins and amino acids is released ammonium ions so that the pH of the media gets wet. Changes in pH occur quickly in a closed environment such as nutrient broth in a tube, thereby inhibiting the growth of microorganisms. To prevent changes in pH, a buffer solution is often added to the media. In general, bacteria grow well at a pH of around 7.0, although they can grow in a pH range of 5.0 - 8.0. To see the effect of pH, bacteria are grown at various pHs.

The increase and decrease in enzyme activity can be influenced by various factors including temperature and pH conditions. pH also affects the speed of enzyme activity in catalyzing a reaction. Each enzyme has an optimum pH where at that pH the three-dimensional structure is most conducive to binding to the substrate. According to Saropah et., Al. (2013) pH strongly influences enzymatic reactions where changes in pH have a direct effect on enzyme ionic groups, thus affecting active enzymes and enzyme conformation. In addition, too large changes in pH above the optimum pH cause enzyme denaturation. Enzymes show maximum activity in a range called optimum pH which is generally between pH 4.5 - 8 (Rosnawita, 2015).

According to (Feliatra, 2016) microbes in the environment of natural habitats need various types of gases such as oxygen, carbon dioxide, nitrogen, and methane. To cultivate microbes in the laboratory, we must pay attention to the needs of a number of atmospheric gases. Based on the response to oxygen, the microbes can be divided into 4 groups, namely; (1) Aerobic microbes; microbes that require oxygen to grow; (2) Facultative anaerobic; microbes that can grow in the atmosphere of free air or without air; (3) Anaerobic microbes; microbes that die when they come into contact with the oxygen and cannot grow in the open air and do not use oxygen to produce energy from biochemical reactions; (4) Microaerophilic microbes; microbes that grow well when there is less oxygen as well as aerobic microbes can use oxygen and produce energy from the chemical reaction process. Unlike aerobic microbes, microaerophilic microbes cannot withstand oxygen levels between 1 - 15%.

Some potential bacterial species are mineralized and solubilized for organic and inorganic phosphorus, respectively. 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 global cycling of organic and inorganic phosphate insoluble soils (Karpagam, 2014).

**Methods**

This practicum activity is carried out with the following objectives; (1) Analyzing the effect of temperature factors on the growth of Escherichia coli bacteria; (2) Analyzing the effect of chemical factors on the growth of Escherichia coli bacteria.

**Time and place**

This practicum was held on Thursday, January 25, 2018, at the Microbiology Laboratory, Makassar State University

**Tools and Materials**

The equipment used in this practicum is a Caliper, test tube, refrigerator, incubator, oven, petri dish, tweezers, enkas, Bunsen burner, bottles, inoculation needles.

In this practicum, materials such as escherichia coli culture, nutrient broth (nb) medium, agar nutrient (na), sterile paper disk, antibiotics (ampicillin, tetracycline, and colsancetine), liquid bath soap (branded Nuvo, dettol, and lifebuoy), chemical solutions (formalin, 70% alcohol, and iodine), aquades, cotton stoppers, aluminum foil, plastic wrap.

**Work procedures**

**Temperature Factor**

- Fill three test tubes with NB medium
- Inoculation of Escherichia coli culture into tubes of 0.5 mL each
- Cover the tube with cotton stoppers and Aluminum foil
- Put a mark on the tube in the form of 5 °C, 30 °C and 50 °C
- Incubate the tube marked 5 °C in the refrigerator
- Incubate the tube marked 30 °C in the incubator
- Incubate the tube marked 50 °C in the oven

**Effect of Chemical Factors**

- Pour NA medium into three petri dishes
- Satisfy paper disks in bottles containing antibiotic suspensions, liquid bath soaps, and chemical solutions, as well as bottles containing distilled water
- Inoculation of Escherichia coli cultures into petri dishes was 0.1 mL each
- Give a mark on the petri dish
Results and Discussion

Temperature factor

| Types of Microbes | Temperature |
|-------------------|-------------|
|                   | 5°C  | 30°C | 50°C |
| Eschericia coli   | -    | ++   | +++  |

Chemical factor

| No. | Image Observation Results | Information | Result |
|-----|----------------------------|--------------|--------|
| 1   | Antibiotics               | Ampicillin   | -      |
|     |                            | Tetracyline  | 1.045 cm |
|     |                            | Colsancetine | -      |
The results of the measurement of the inhibition zone formed around the paper disk with antibiotics in the form of tetracycline are 1.04 cm on the right side and 1.05 cm on the left side so that it is obtained, \( \frac{1.04\, \text{cm} + 1.05\, \text{cm}}{2} = 1.045\, \text{cm} \)

**Chemical Solvent**

The results of the measurement of the inhibition zone formed around the paper disk with a chemical solution in the form of formalin are 4 cm on the right side and 3.02 cm on the left side so that it is obtained, \( \frac{4\, \text{cm} + 3.02\, \text{cm}}{2} = 3.51\, \text{cm} \)

In this practicum, an analysis of the effect of temperature and chemical factors on microbial growth is carried out. The first activity carried out was to analyze the temperature factor on microbial growth. This activity used a test tube and 9 mL nutrient broth (NB) as a microbial growth medium. The sample used was Escherichia coli bacterial culture. The sample is then placed in a test tube containing a medium for subsequent incubation. The incubation process is carried out in the refrigerator for 5 °C, in the incubator for 30 °C, and in the oven, for 50 °C. Each tube is placed in a different place to distinguish the temperature of the incubation. After the incubation process, it can be seen that the tube which is placed in the refrigerator, is clearer when compared to the other tubes. This happens because at low temperatures the bacteria are not actively metabolized. While the tube is placed in the oven, it looks very murky. Turbidity occurs due to the number of microorganisms that die because it cannot grow in high-temperature environments. The tube incubated in an incubator with a temperature of 30 °C looks rather turbid, it shows that there are bacteria that grow in the medium optimally.
In the activity of analyzing the influence of chemical factors on microbial growth materials are used in the form of several antibiotics, several brands of liquid bath soap, and some chemical solutions. The first petri dish is a petri dish that has been given several paper disks each from antibiotics namely Ampicillin, Tetracycline, Colsancetine, and distilled water as controls. In the petri dish, there is a zone of inhibition, which is the zone around the paper disk that is not overgrown by bacteria. The zone is on a paper disk with antibiotics in the form of Tetracycline. After being analyzed, the diameter of the circle formed was 1,045 cm. Meanwhile, in Petri dishes with liquid bath soap, there is no inhibiting zone. As for the petri dish which was given a paper disk from a chemical solution in the form of formalin, 70% alcohol, and iodine, a bacterial free zone was seen around the formalin paper disk. The zone is about 3.01 cm in diameter.

Chemical factors that have inhibition based on the results of this practicum are Tetracycline and formalin. In this experiment, no type of liquid bath soap was found that inhibited the growth of E. coli. This can occur because of an error in the experimental procedure. It can be said that the other ingredients used do not affect the growth of E. coli bacteria because it does not inhibit its growth.

**Conclusion**

Based on the results and data analysis, it can be concluded that the Escherichia Coli bacteria can grow optimally in the temperature range of 30 °C. At low temperatures, the bacteria are inactive, whereas at relatively high temperatures the bacteria cannot survive. Formalin has a strong inhibitory effect on the growth of Escherichia coli bacteria, as well as Tetracycline antibiotics which can inhibit the growth of E. coli bacteria.

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