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Abstract. Many schools do not organize the biological practice with the topic of bacterial inoculation and Gram staining, especially remote schools in the archipelago due to the difficulty of obtaining bacterial growth medium (Nutrient Agar/NA). This study aims to find alternative sources of nutrients used in NA medium that can be used by teachers in remote areas of the archipelago to be able to carry out bacterial and Gram staining breeding practicum. This research consisted of several stages, namely: (1) sample preparation; (2) manufacture of NA control medium; (3) the production of an alternative medium by the treatment of the use of Layang fish extract (*Decapterus russelli*) with peptone and without peptone; (4) bacterial inoculation with three different sources; and (5) the calculation of bacterial colonies. The data obtained were analyzed by using Descriptive Analysis. The results showed that the medium of "Narik Layang" using the extract of Layang fish (*Decapterus russelli*) could be used as an alternative to NA medium that using beef extract. Based on these results, the biology teacher can use the extract of Layang fish as a substitute for beef extract in the manufacture NA medium to carry out bacterial inoculation and Gram staining practicum.

1. Introduction

One of the topics of biology in high school is bacteria and eukarya [1]. The material requires that one of the skills that are important to be mastered by students is bacteria propagation techniques. These techniques require a bacterial growth medium. Medium for bacterial growth is a material consisting of a mixture of nutrients that bacteria need for growth. Bacteria utilize the nutrients of the medium in the form of small molecules that are assembled to make up the cell components as well as essential for cell development and reproduction.

The medium that is often used to breed bacteria is the NA (Nutrient Agar) medium. Medium NA can be obtained in the form of finished products, but the price is quite high besides that not all regions sell the medium, especially the areas in the countryside of the remote areas. This cause the biology teachers

in the area do not do practicum about the bacteria breeding, to overcome this it is necessary alternative by making your own NA medium for bacteria breeding.

Based on its composition, the NA medium consists of beef and peptone extracts that act as a source of nutrients, agar, and water [2, 3]. Meat extract used in the manufacture of this medium is usually a beef extract whose price tends to be more expensive and limited availability in remote rural areas, especially the islands. Therefore, other sources of nutrients that can be used as an alternative to beef so that teachers in the archipelago, especially in Southeast Sulawesi region can make NA medium with low cost and easily accessible materials is necessary.

Fish is a natural commodity in the archipelago. Murniatin [4] suggested that fishery catches in Southeast Sulawesi are dominated by pelagic fish such as Cakalang, Kembung, and Layang fish. Layang fish, in particular, ranks highest regarding both inventory and sales, since most people consume this type of fish. The fact is traditional fisherman earn more from Layang fish than other fish sales from the catches. According to Nontji [5], Layang Fish is one of the essential small pelagic fisheries communities in Indonesia. Fish belonging to this Carangidae tribe can live together. The size is about 15 cm although some can reach 25 cm. The characteristic of flying fish is that there is a small fin behind the dorsal and anal fins and there is thick lateral fin on the lateral line. Layang fish meat has a high protein content which is an essential source of nutrients for bacterial growth [6]. Due to its abundance and lower price, the Layang fish has the potential to be a source of protein in the manufacture of the growing bacterial medium. The objective of this research is to find alternative sources of nutrients used in NA medium that can be used by teachers in remote areas of the archipelago to be able to carry out bacterial and Gram staining breeding practicum.

2. Materials and Methods

This research was conducted at the Education Unit Laboratory of Biology, Faculty of Teacher Training and Education, Haluleo University, Kendari, Southeast Sulawesi. *Enkas* (a different form of Laminar Air Flow) was cleaned by spraying alcohol first and then put the bunsen burner with a flame in it with the aim of creating an air flow that can kill contaminants. Glassware were wrapped with paper and then tightened by using a rubber band, the test tube and Erlenmeyer tube mouth were corked first using cotton. Layang fish were washed until clean and were separated for the meat using a knife.

Control medium using beef extract was prepared following these stages: (1) Weighed 500 grams of beef using Ohaus scales; (2) Incorporate the scales into a pot together with 800 ml of distilled water; (3) Cooking the meat for ± 30 minutes; (4) Add the distilled water until the extract volume becomes 1000 ml then beef extract is ready for use. Layang fish medium was prepared by using this subsequently method: (1) Weigh the sample meat as much as 500 grams using Ohaus scales; (2) Put the sample into a saucepan together with 800 ml of distilled water; (3) Cooking the fish using a stove for ± 20 minutes; (4) Add the distilled water until the extract volume becomes 1000 ml then the fish extract is ready for use.

After the fish extract is ready then weigh for 15 gram of agar and peptone as much as 5 gr then mix the medium with the following steps: (1) Divide the existing fish extract into two parts, each into a beaker, one part (700 ml) used to dissolve the agar and the rest (300 ml) to suspend the peptone; (2) Insert the peptone that has been weighed into a beaker containing 300 ml of lobster meat extract and stirring until homogeneous; (3) Put the weighed agar into a beaker containing 700 ml of fish extract and stirring using a stirring rod on the hot plate until it is homogeneous; (4) Mix the contents of the two beakers and stirring them until homogeneously forms the finished NA medium; (5) Pouring the finished medium into Erlenmeyer flask and then closing the pumpkin's mouth with cotton and the medium ready to be sterilized. After the apparatus and materials are prepared, then sterilize them both using the autoclave. The material and tools were sterilized for 15 and 30 minutes, respectively at a pressure of 15 psi with a temperature of 121^{o}C.

After sterilizing the tools and ingredients then inoculate the bacteria on the plate using a pour plate and then counting the growing colon on each cup. According to Waluyo [7], bacterial growth is the increase in the number of organisms that make up the population or culture. Based on this, one way to

measure the growth of a type of bacteria is to calculate the number of bacteria both the number of cells and the number of bacterial colonies that grow on the medium provided. The method used to calculate the number of bacteria was as follows: (1) Dilution of the sample until the dilution of 10^{-5} for samples of stagnant water and fish gills then dilution 10-10 for yogurt; (2) Inoculating the last 3 dilutions to a new medium specifically for the yogurt sample inoculated up to the previous 5 dilutions of the cup; (3) Incubating the sample for 2 x 24 hours then calculate the growing colony using the SPC (standard plate count) technique.

3. Results and Discussion

The results showed that bacteria could grow on each medium (control, fish extract without peptone and fish extract with peptone) (table 1).

Table 1 Research results regarding the number of bacterial colonies growing on the medium NA

Medium NA	Source of bacteria	Number of colonies (CFU/ ml) repeat			Average (CFUs/ ml)
		1	2	3	
Beef extract	Yoghurt	5.9×10^7	8.5×10^7	5.8×10^7	6.7×10^7
	Fish extract	5.9×10^7	4.3×10^7	7.2×10^7	5.8×10^7
	Dirty water	2.6×10^6	2.1×10^6	3.4×10^7	1.3×10^7
Fish extract with peptone	Yoghurt	2.4×10^9	2.2×10^9	2.0×10^{10}	8.2×10^9
	Fish extract	4.0×10^7	$<3.0 \times 10^7$ (2.2×10^7)	4.1×10^7	3.4×10^7
	Dirty water	3.3×10^5	2.6×10^5	4.3×10^5	3.4×10^5
Fish extract without peptone	Yoghurt	4.6×10^7	3.1×10^8	2.8×10^9	1.1×10^9
	Fish extract	4.5×10^7	4.6×10^7	4.0×10^7	4.4×10^7
	Dirty water	6.9×10^5	4.5×10^5	1.9×10^5	4.4×10^5

Table 1 shows that the highest average number of colonies was found in NA medium using Layang fish extract without peptone inoculated with a source of bacteria originating from the yogurt while the average number of bacterial colonies was lowest in NA medium which uses Layang fish extract with inoculated peptone with a bacterial source derived from dirty water. Also, there is a tendency of a high number of colonies in NA medium that uses beef extracts compared to those using Layang fish meat extract on fish meat and source of dirty water while in yogurt source the opposite is the lowest number of colonies on NA medium using beef extract. The average number of bacterial colonies found in the NA medium types are shown in figure 1.

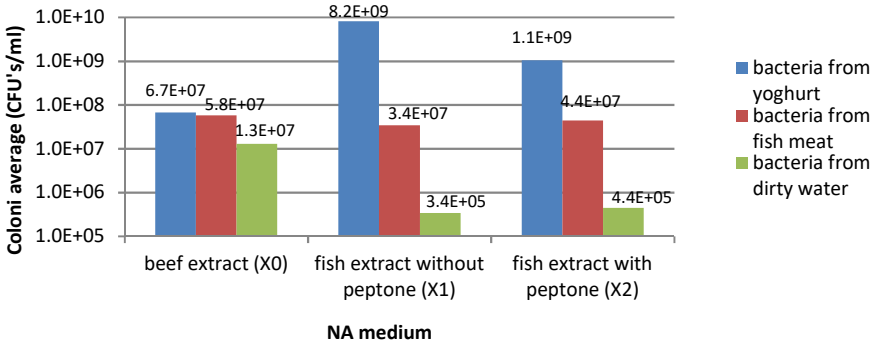

Figure 1. Graph of relation between NA medium type to average amount bacterial colonies

Figure 1 shows that the highest average number of colonies is found in NA medium using Layang fish extract without peptone for the source of bacteria originating from yoghurt, *ie* 8.2 x 10^9 CFU/ml while the average number of colonies the lowest is also found in NA medium using Layang fish extract without peptone to source bacteria derived from sewer water. Also, the graph also showed that in addition to the sources of the yogurt bacteria, the NA medium using beef extracts showed the highest number of colonies of 5.8 x 10^7 CFU/ml for the origin of fish meat bacteria and 9.2 x 10^6 CFU/ml for the source of bacteria sewage.

Bacteria require various sources of bacteria in their growth, including carbon, nitrogen, sulfur, phosphorus, minerals, and vitamins. Carbon is essential in the growth of bacteria one of them as the builder of cell components that require carbon framework for example carbohydrates and lipids. Nitrogen is a major component of proteins and nucleic acids, which is about 10% of the dry weight of bacterial cells. Nitrogen may be supplied in different forms, and bacteria vary in their ability to assimilate nitrogen [8].

Sudarmadji *et al.,* [9] explained that protein has a feature that contains N elements in addition to C, H, O (as well as carbohydrates and fats), sometimes Fe and Cu (as complex compounds with proteins). Sulfur is a component of many organic substances cells. Sulfur forms part of the structure of several coenzymes and is found in several side chains of cysteine and methionyl protein. Phosphorus is needed as a component of ATP, nucleic acids and some coenzymes such as NADP and flavin. Also, many metabolites, lipids (phospholipids) are phosphate groups (PO_4^{3-}) while large amounts of minerals are needed for enzyme function. Magnesium ions (Mg^{2+}) are also found in porphyry derivatives of magnesium in chlorophyll [8].

All living things including bacteria need vitamins (unique organic compounds essential for growth). Most vitamins function to form substances that activate enzymes - substances that cause chemical changes [10]. According to Winarno [11] vitamin is an organic molecule that is indispensable for the process of metabolism and growth. Vitamins cannot be made in sufficient quantities because they are obtained from outside the body. This result shows that the NA medium using the meat extract of Layang fish both without peptone and using peptone has the potential as an alternative to replace the NA medium that uses beef extract to save production costs and can facilitate the lab inoculation of bacteria.

4. Conclusion

There was no significant difference between the number of bacterial colonies grown in NA medium using beef extract and medium NA using Layang (*Decapterus russeli*) fish extract either not using peptone or using peptone. Medium NA using fish meat extract (*Decapterus russelli*) can be used as an alternative to medium NA using beef extract.

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