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DIFFERENCES IN THE NUMBER OF BACTERIA AND CHARACTERISTICS IN THE ENDO AGAR MEDIUM IN DISHWASHING SPONGES WITH LONG USE

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Abstract

Introduction: Sponge is one of the common scrubbing tools found in Indonesia as a dishwasher. Soaking adishwashing sponge in a wet container can make the sponge moist and become an ideal place for microorganisms to grow such as *Escherichia coli* bacteria that can grow on a wet surface. Some factors that still often occur in the community are in users of dishwashing sponges that exceed 3 days of use and are not replaced, this can make *Escherichia coli* breed in the sponge. Food can be contaminated caused by dishwashing sponges, which are transmitted through cutlery. A case study in the United States, after 1,000 kitchen washcloths and washing sponges that had been collected, obtained 10% where the objects contained *Salmonella* and *Escherichia coli*

Method: Bacterial identification is carried out on 10 dishwashing sponges used for 1 week and 2 weeks

Results:. The characteristics of colonies obtained in BHIA media in sponge samples of 1 and 2 weeks are circular, with a margin of entire, opaque, white-gray or milky-white with a smooth texture, in Endo Agar media grow colonies of red to metallic green color, Simmon citrate agar negative, glucose and lactose positive tests.

Conclusion: Based on the results of the ALT calculation study, it was found that the dishwashing sponge used 2 weeks more than the dishwashing sponge used for 1 week. Alt calculation with a duration of use of 2 weeks obtained results Sp1 = 6.0 ×107, Sp2 = 4.7×106, Sp3 = 7.2×107, Sp4 = 6.0 ×105, Sp5 = 6.6×107. While the calculation results of ALT 1 week are Sp1 = 4.6 ×107, Sp2 = 1.6×106, Sp3 = 4.8×107, Sp4 = 5.4 ×105, Sp5 = 4.5×107. The characteristic of bacteria that are suspected to grow on dishwashing sponges is *escherichia coli* bacteria.

Key words : Brain Heart Infusion Agar , Dishwashing sponge, Endo Agar, Food borne disease, Total Plate Count (TPC).

INTRODUCTION

Sponge is one of the common scrubbing tools found in Indonesia as a dishwasher. Soaking a dishwashing sponge in a wet container can make the sponge moist and become an ideal place for microorganisms to grow such as Escherichia coli bacteria that can grow on a wet surface. If the sponge is not dry or damp because the sponge is soaked in a container, it can be an ideal place for bacteria to grow (Gaffar *et al*., 2014). Storing a good dishwasher is something that really needs to be considered. Soaking a dishwashing sponge in a wet container can make the sponge will be damp and become a nest for breeding microorganisms. According to Kalem (2019) some factors that still often occur in the community are in users of dishwashing sponges that exceed 3 days of use and are not replaced, this can make *Escherichia coli* multiply in the sponge.

Food can be contaminated caused by dishwashing sponges, which are transmitted through cutlery (Rossi *et al*., 2012). Eating or drinking utensils that have been contaminated by bacteria can cause food borne disease (Fadhila *et al*., 2017). Food borne disease is a disease that causes poisoning of the body and is caused by consuming food that has been contaminated with various microorganisms. Some microorganisms that can cause Food borne disease are *Escherichia coli* bacteria (Motarjemi *et al*., 2006). Generally, tableware has been regulated by the Regulation of the Minister of Health of the Republic of Indonesia No. 715 / MENKES / SK / V / 2003 which states about, that in eating or drinking utensils must not contain bacteria more than 100 colonies / cm2 on the surface and there are no *Escherichia coli* bacteria. According to the Regulation of the Minister of Health of the Republic of IndonesiaNo. 1096/MENKES/PER/VI/2011 said that regarding sanitary hygiene, eating and drinking utensils must not have *Escherichia coli* germs or other germs. According to the Decree of the Minister of Health No. 715,

said food sanitation hygiene is an important thing that can control equipment, food, and factors of people that can cause disease or health. Gaffar *et al* (2014) in identifying bacterial growth in dishwashing sponges by soaking the sponge overnight, *E.coli* bacterial growth was obtained. Based on previous research by Andini *et al* (2021) obtained *E. coli* bacteria in dishwashing sponges used for 6 weeks. The studyconducted previously by researchers came from Arizona, united states, after 1,000 kitchen washcloths and washing sponges that had been collected, obtained 10% where the objects contained *Salmonella* and *Escherichia coli* (Riboldi G., 2002).

METHOD

The research used in this study is an experiment using 2 variables, namely Independent and Dependent. The intervention or treatment in this study was the duration of use of dishwashing sponges, namely 1 week and 2 weeks. This study conducted an examination on the dishwashing sponge to see the comparison of the number and characteristics of bacteria. This research was conducted from February to March 2022. The research was conducted at the Bacteriology Laboratory of the Tingi School of Health Sciences, Mitra Keluarga. The samples used in this study were 5 dishwashing sponges with a duration of use of 1 mingu and 5 dishwashing sponges with a duration of use of 2 weeks.

Samples that have been used for 1 week and 2 weeks are taken using a sterile cotton swab by means of, The whole cotton swab is dipped first into the sterile aquadest. Sampling by rubbing the cotton swab rotates so that the entire surface of the cotton is in contact with the surface of the sample. (Ramadhani and Wahyuni., 2020). Then carry out the titular dilution by means of samples containing bacteria are put intothe first dilution tube (1/10 or 10^{-1}) aseptically. After the sample enters, it is then homogenized, then take 1 ml from retailers 10^{-1} using a measuring pipette then transfer it to dilution 10^{-2} aseptically and then dihomogenized. The transfer is continued until the last dilution tube, which is 10^{-5} using a sterile pipette tip (Ramadhani and Wahyuni., 2020).

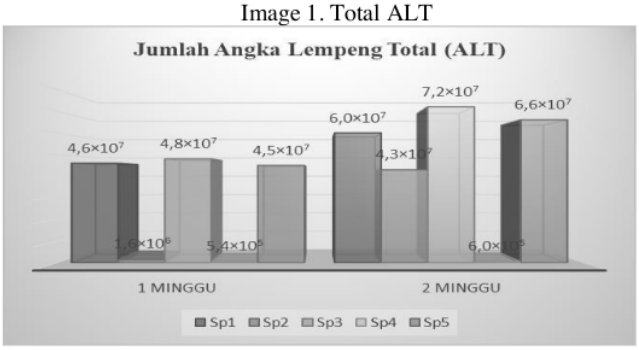
Then planting colonies on BHI-A media by pipette 100 µl at a dilution of 10-2 to 10-5, then incubated in an incubator with a temperature of 370C for 24 hours. After planting on BHI-A media, as many as 10 different colonies using ose then take and plant on Endo Agar media and then incubated in an incubatorwith a temperature of 37oC for 24 hours. Colonies of bacteria growing on BHI-A media after 24 hours are calculated. manually using a colonl counter tool. Calculating the number of bacterial colonies by means, the cup is placed on a bright flash or LED. After that, do gram coloring by the way, the violet kistal is picketed and then color the preparation evenly, then wait up to 1 minute. After waiting for 1 minute rinse the preparation under running water. Next, pipette iodine then drip on the preparation until evenly distributed, wait up to 1 minute. After that washing with running water and then removing the color with alcohol. Recoloring with safranin then waited for 1 minute, after 1 minute rinse the preparation with running water then dried (Sujaya, 2016).

RESULTS

On a sample of dishwashing sponges with a duration of use of 1 week. The characteristics of colonies growing in BHI-A media are dominated by colonies with a circular shape, with an entire margin, the colony cannot be translucent (opaque), white-gray or milky-white with a smooth texture, as in the table. According to Indrayati and Fatimah (2018) colony characteristics in table 4.1 below, it is suspected to be a colony characteristic of the bacteria *Escherichia coli*.

Sample dishwashing sponge with a duration of use of 2 weeks. The characteristics of colonies growing in BHI-A media are dominated by colonies with a circular shape, with a margin of entire, the colony cannot be translucent (opaque), gray-white or milky-white with a smooth texture. According to Indrayati and Fatimah (2018) colony characteristics are suspected to be colonies of *Escherichia coli* bacteria.

Image 1. Total ALT



The result of the number of colonies on dishwashing sponges used for 1 week is less than that used for 2 weeks and can be seen in figure 1. After obtaining a culture colony from BHI-A media, then inoculation is carried out on Endo Agar media to find out the nature of the colony growing in BHI-A media, whether the colony can ferment lactose or not.

Table 1. Characteristics of colonies in Endo Agar media

No	Sample code	Colony color
1	3	White
2	4	does not grow
3	5	Red
4	6	metallic red

Colonies in scratches coded 5 and 6 with culture colonies obtained from BHI-A media are then streaked in Endo Agar media, which is thought to be a characteristic colony of *Escherichia coli* bacteria, because colonies are round, convex, entire and gray-white or milky-white in color. In the results of inoculation in Endo Agar media, there are growing colonies of red color until there is metallic green. *Escherichia coli* bacteria can grow on Endo Agar media with the characteristics of colonies of red to metallic dark red color (Indrayati and Fatimah, 2018).

Bacterial colonies that grow with the characteristics of colonies of metallic red to green color in Endo Agar media are taken and inoculated on SCA media to test whether colonies in Endo Agar media can grow on SCA media.

Table 2. Characteristics of bacteria on biochemical tests

No	Sample code	Result		
		Simon Citrat	Glucose	Lactose
1	5	-	+	+
2	6	-	+	+

The results on the citrate test obtained negative results, and in glucose and lactose tests, positive results were obtained. Then in the gram staining test results, the results of red-colored bacteria or gram-negative bacteria were obtained. Different colony numbers can be caused by discharging factors with a longer period of time. According to Kalem (2019) several factors that cause the growth of Eschericia coli bacteria in sponges are caused by use that exceeds 3 days of use and are not replaced with new ones. According to Ermia (2021) the high number of bacteria is due to poor washing stages, poor storage of hygiene tools.

DISCUSSION

The result of the number of colonies on dishwashing sponges used for 1 week is less than that used for 2 weeks and can be seen in figure 1. In table 1 scratches with code 4 are seen colonies do not grow on the medium, on strokes with code 3 are white,5 and 6 are seen the growth of red to metallic red-colored bacteria. Colonies in scratches coded 5 and 6 with culture colonies obtained from BHI-A media are then streaked in Endo Agar media, which is thought to be a characteristic colony of *Escherichia coli* bacteria, because colonies are round, convex, entire and gray-white or milky-white in color. In the results ofinoculation in Endo Agar media, there are growing colonies of red color until there is metallic green. *Escherichia coli* bacteria can grow on Endo Agar media with the characteristics of colonies of red to metallic dark red color (Indrayati and Fatimah, 2018).

The observation results obtained in the citrate test were negative in colonies in Endo Agar media which had a characteristic red to metallic green color which was suspected to be Escherichia coli showed negative results by not experiencing discoloration in the sirat test media. *Escherichia coli* bacteria do not use citrate as a source of carbon which is indicated by the absence of discoloration in the citrate test media. Simmon Citrate In order to function as a testing medium to determine the properties of bacteria, the bacteria can ferment citrate or not as a source of carbon (Rahayu and Gumilar, 2017).

The results of the bacterial fermentation test on glucose and lactose media obtained positive results with the marked gas formation in the durham tube. According to Harley (2012) the occurrence of discoloration from red to yellow is caused by the presence of phenol red indicators that blind the occurrence of acid formation in this carbohydrate fermentation test medium. According to Jorgensen (2015) *Escherichia coli* bacteria can ferment glucose and lactose which is in accordance with the results in this study. According to Mahon (2015) bacteria can be able to ferment carbohydrates, so there is a glycolysis process that can produce the final product, namely in the form of pyruvate and converted so that acid formation occurs.Then the acid is converted into H2 and CO2 through an enzyme mechanism, namely hydrogen lyase, sothat it can produce gases that are formed in the durham tube.

Based on the results above on the staining of Gram bacteria A shows a red color, indicating that the yaang tumbu bacteria are Gram negative. This *Echerichia coli* bacteria is a Gram-negative bacterium, whengiven violet crystal staining then declorized with alcohol will fade or disappear in gram-negative bacteria and when dripped the counter-coloring agent, namely safranin, will retain its color, which is red, this is because the cell wall of the bacteria has a different chemical structure. Gram-negative bacteria have only one layer of peptidoglycan on the cell wall bound to the outer membrane lipoprotein. Gram-negative bacteria contain teicoic acid because they only contain a small amount of peptidoglycan, so the cell wall is more susceptible to mechanical damage.

CONCLUSION

Based on the results of the ALT calculation study, it was found that 2-week dishwashing sponges were more than dishes-washed sponges used for 1 week with a sig value of 0.002. ALT calculation with a duration of use of 2 weeks obtained results Sp1 = 6.0 ×107, Sp2 = 4.7×106, Sp3 = 7.2×107, Sp4 = 6.0 ×105, Sp5 = 6.6×107. While the calculation results of ALT 1 week are Sp1 = 4.6 ×107, Sp2 = 1.6×106, Sp3 = 4.8×107, Sp4 = 5.4 ×105, Sp5 = 4.5×107. The characteristic of bacteria that are suspected to grow on dishwashing sponges is *Escherichia coli* bacteria

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