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The Identification of Microbial Characteristics and Number of Colonies Isolated From River Water in the Region of Mulyoagung Singgahan Tuban

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Abstract

River is one source of fresh water flowing from upstream to downstream which has a lower surface of the earth's surface and the flow will lead to sea, lakes, swamps, and others. Nglirip River is one of the Rivers in the village of Mulyoagung Singgahan District of Tuban surrounded by the agricultural sector around the population and it is usually used by people as the fulfilment of daily domestic activities. This study aims to identify the characteristics of microbial colonies were isolated from the River of Nglirip and identify the difference in the number of microbial colonies. The dilution method used was an experimental laboratory. Data were analyzed descriptively and quantitatively with the Mann-Whitney. The result of conducted research using the pour plate method 171 cfu bacterial colonies obtained from the dilution of 10^{-5} and 116 cfu bacteria colonies of dilution 10^{-6} and after Mann-Whitney test, there was no significant difference of colonies number of microbes between dilution 10^{-5} and 10^{-6} dilutions. The conclusion is that the characteristics of microbial colonies were isolated from River water Nglirip Tuban area Mulyoagung majority are round, white colored milk and belonged to Gram-negative bacteria. And there was no significant difference in the number of colonies of microbes in different dilutions.

Keywords: Nglirip River; Pour cup method; Gram staining; Gram-negative bacteria

Background

Indonesia is a rich country of water resources which can be seen from the potential availability of surface water especially River. River is one source of fresh water flowing from upstream to downstream which has a lower surface of the earth's surface and the flow will lead to sea, lakes, swamps, and others.

Water has a major role in the metabolism mainly because it has an important role in digestion, food absorption, and transport nutrients in the body [1]. Water also can play a role in the spread of bacteria, so that when water contaminated with this bacteria for human consumption, it will have implications for the human health [2].

Nglirip River is located in the village of Mulyoagung, District Singgahan, Tuban precisely in Jojogan village. Nglirip River is sourced from wellspring Krawak and it has canal of Nglirip waterfall [3]. Nglirip River used as a source for people around meeting the needs of water daily for domestic use, irrigation farming, and in some people often bathe there. On behalf of the importance, the water of Nglirip River must be into the first category which means the water does not contain hazardous materials either from a chemical or biological. The parameter of indication of bacteriological water quality is the basis. Due to water contamination by pathogenic bacteria can be the cause of environmentally-based-diseases such as diarrhea [4,5].

The isolation of bacteria is one of the techniques of growing bacteria in a medium that is not its origin place. The media in the isolation microbes takes an important role in the growth of microbes [6]. Microbial cells are still alive, will be able to grow and develop to form colonies in the same type that can be calculated directly without using a microscope. Observation using microscope done by gram staining in advance to determine the morphology and type of bacteria.

The result of research conducted by [1] in India showed that gram-negative bacteria *E. coli* bacteria in the water was the majority of the lake. The bacteria *Escherichia coli* which is gram-negative bacteria are rod-shaped and classified in the class of coliform bacteria. Wherein the bacteria are generally found in water contaminated by feces or feces of living creatures, especially mamalia. This study aims to identify the characteristics of microbial colonies were isolated from River water in the village Nglirip Mulyoagung Singgahan District of Tuban and to determine the differences of microbial colonies number on the different dilutions.

Method

Determination of point sample

The sampling method is purposive sampling which is the procedure performed by some of the considerations made by the researcher [7]. The consideration is such suspecting source that can represent throughout of the Nglirip River. The location of sampling can be shown in (Figure 1) [8]. From the map image which has a scale of 1: 1000 m is the location of water sampling Nglirip River is marked with a round symbol and green. Yellow symbols indicate areas or regions around the Nglirip River.

Sample preparation

The sample obtained from Nglirip River was added to a glass jar that had been sterilized and placed inside boxes containing ice to be brought to the laboratory for examination. The dilution was performed stratified, sample dilution began from diluent 10^{-1} then continued dilution used in this study was 10^{-5} and 10^{-6}. The 10^{-1} dilution was done by taking a 1 ml water sample added by 9 ml of water aquades so on and the 10^{-5} dilution was done by taking 1 ml of sample dilution 10^{-4} added by 9 ml of distilled water and dilution 10^{-6} by taking 1 ml of sample dilution 10^{-5} added by 9

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ml of distilled water.

Procedure

The medium used was a nutrient agar medium (NA) that has solidified on the cup petri, it was around 1 ml from 10^{-6} and 10^{-5} dilution. Incubation growth of microbes performed for 24 hours at room temperature. After that microbial colonies were observed in terms of color and shape.

The gram staining was done by taking the size of needle use microbial colonies and then performed gram staining and observed under a microscope. The observed parameters were the shape and type of bacteria that were observed under a microscope. The Observation was conducted within 1 × 24 hours. Parameter observations include color colonies, the number of colonies, the bacteria form colonies and groups with the Gram stain.

Result and Discussion

The data was calculated by the number of microbial colonies using cup count method and presented in (Table 1).

To test whether there was any difference between the amount of microbial colonies of dilution 10^{-6} and 10^{-5}, the Mann-Whitney test was conducted. This test was an alternative to the t test for two independent samples. Mann-Whitney test was done because in this study, the amount of data was limited (Table 2).

From the ranks table, it can be obtained information that the number of ratings data on the number of microbial colonies at 10-5 dilution is 11.50 and the number of ratings data on the number of microbial colonies at 10-6 dilution is 9.50 (Table 3).

So in conclusion, there is no difference in the number of significant microbial colonies between dilutions 10^{-5} and 10^{-6} dilutions (Table 4 and 5).

The results of bacteria cultivation on agar media can be seen in (Figure 2). The black arrows indicate one of the microbial colonies growing on an agar medium. Gram staining results can be seen in (Figures 3 and 4).

Discussion

The isolation of microbes from the River Nglirip was cultured in media Nutrient Agar (NA). Media NA is also commonly used for breeding media microbes isolated from the water and it is very easy to freeze. Observation of morphological characteristics of microbial breeding result is milky white with different sizes and different amounts. On dilution 10^{-5} and 10^{-6} dilution had the same form microbial colonies, those were small round spots or round, large round and curved at the edge of the cup. Those had different amounts of each dilution was in the form of microbial colonies are small round at 10^{-5} dilution amounted to 161 colonies and dilution 10^{-6} amounted to 109 colonies of microbes. For the large round-shaped colonies on 10^{-5} dilution amounted to 9 colony mikroba. Though at 10^{-6} dilution amounted to 6 colonies of microbes. Microbial colonies curved shape between each dilution amounted to the same that each one colony of microbes. At 10^{-5} dilution, the number of colonies of microbes more than 10^{-6} dilution due to the high dilution could affect the amount of microbes in it. The higher dilution, the fewer microbes were detected or that of the sample. One of the goals for the thinning was to reduce the density of the microbes in the sample. From the results Mann-Whitney Test was no significant difference between the dilution 10^{-5} dilution 10^{-6} for judging the results were only slightly adrift because of only one level of dilution. According in the identification of bacterial colonies characteristic it is essential that includes shape, size, color and biochemical tests.

The Gram staining aims to identify groups of bacteria among gram-positive and gram-negative. At the gram stain using crystal violet, safranin, iodine and alcohol. Crystal violet dye is a major to be bound by

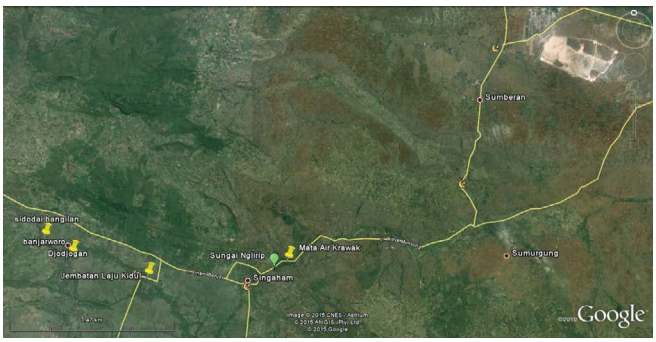

Figure 1: The map of collecting sample.

Characteristics Number	Colony Color	Colony Shape	Colony Number
Dilution 10^{-5}	Milky White	Small round	161 cfu
		Big round	9 cfu
		Curved	1 cfu
Dilution 10^{-6}	Milky White	Small round	109 cfu
		Big round	6 cfu
		Curved	1 cfu

Table 1: The data of identification result of microbial colonies and the calculation of total colonies microbes.

Colony Number	dilution 0,00001	3	3.83	11.50
	dilution 0,000001	3	3.17	9.50
	Total	6		

Table 2: To test whether there was any difference between the amount of microbial colonies of dilution 10^{-6} and 10^{-5}, the Mann-Whitney test was conducted.

	Colony Number
Mann-Whitney U	3.500
Wilcoxon W	9.500
Z	-0.443
Asymp. Sig. (2-tailed)	0.658
Exact Sig. [2*(1-tailed Sig.)]	0.700[a]

a. Not corrected for ties.
b. Grouping Variable: Karateristik
Interpretation Result Analysis
Table 3: Test Statistics[b]

	Characteristics	N	**Mean Rank**	**Sum of Ranks**
Colony Number	dilution 0,00001	3	3.83	11.50
	dilution 0,000001	3	3.17	9.50
	Total	6		

Table 4: Ranks

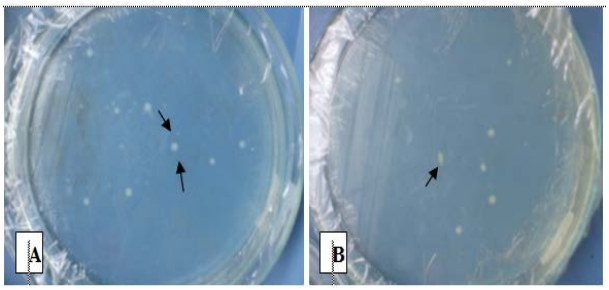

Figure 2: The result of breeding microbes on Media NA with (A) Dilution 10^{-5} and (B) Dilution 10^{-6} The next method was a gram staining method to determine the type and shape of the cells of bacteria isolated from River water Nglirip.

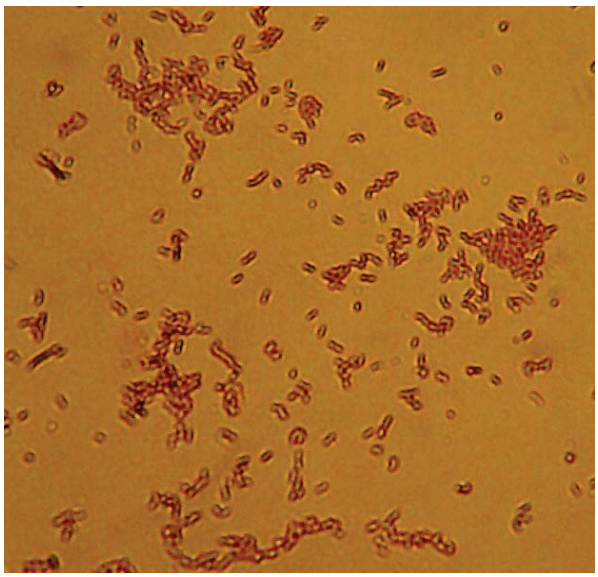

Figure 3: The Results of Gram stain Microbes in dilution 10^{-5} with a magnification of 1,000 times.

	Jumlah Koloni Mikroba
Mann-Whitney U	3,500
Wilcoxon W	9,500
Z	-,443
Asymp. Sig. (2-tailed)	,658
Exact Sig. [2*(1-tailed Sig.)]	,700[a]

a. Not corrected for ties.
b. Grouping Variable: Karateristik

Table 5: From the above Table Test Statistics can be known Asymp. Sig. (2-tailed) of 0.658 greater than is 0.05 then the decision is that we get the same also, that H0 is accepted.

bacterial peptidoglycan and will be retained on gram-positive bacteria. Iodine is a gram staining material that serves to attach the main dye in bacteria and forms a complex iodine-violet. Alcohol is used as a laxative ingredient primary colors so as to be able to bind to bacterial secondary colors are safranin. Safranin as a secondary dye [9].

The results from staining gram, gram-positive bacteria would be obtained if these microbes could withstand complex primary colors purple crystal violet until the end of the procedure the cells appear dark purple or blue. As for the gram-negative bacteria, organisms will

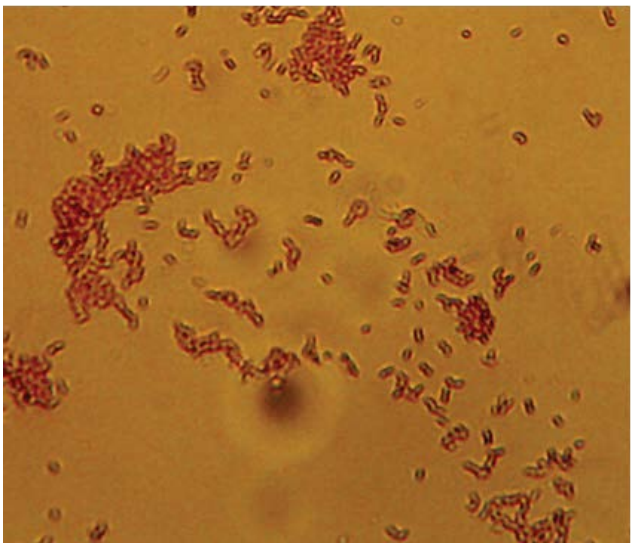

Figure 4: The Results of Gram stain Microbes in dilution 10^{-6} with a magnification of 1,000 times.

lose a complex of crystal violet purple color at the time of flushing with alcohol but then stained by safranin that match the color of the cells appear red or pink. The basic difference between gram-positive and gram-negative bacteria is the component of the cell wall. Gram-positive bacteria have a single membrane coated with a thick peptidoglycan. Meanwhile, gram-negative bacteria, peptidoglikannya thin layer so it can absorb the secondary colors [6,10].

The obtained microbial colonies gram staining results and observation under a microscope had features such as red and rod-shaped. These characteristics indicated that the bacteria including gram-negative bacteria. Presence of gram-negative bacteria in the River, proving that Nglirip River polluted by manure or feces and suspected to contain pathogenic bacteria. Bacterial pathogens were waters, most of whom are of the type of fecal coliform bacteria [1,2,11-13]. In general, fecal coliform bacteria that existed on the waters are due to the type of *Escherichia coli* can survive outside the digestive system. The presence of these bacteria in the water is not harmful, but indicates the presence of other pathogenic bacteria [14]. There are several strains of *E.coli* that if it gets into the digestive system will result in stomach diseases such as diarrhea [10]. Therefore, polluted River water and detected the presence of bacteria indicators, unfit to be used for drinking water as well as for daily needs in domestic activities.

Conclusion

Based on the results of research and discussion, it can be concluded that the characteristics microbial colony isolated from the water of Nglirip River, Mulyoagung Singgahan Tuban area is milky white, spherical shape and the majority belonged to Gram-negative bacteria that form the stem. And there was no significant difference between the number of microbial colonies 10^{-5} dan dilution 10^{-6}.

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