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Potention of Black Sea Cucumber Symbion Bacteria (*Holothuria atra*) as a Lactic Acid Source

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Abstract:

Probiotic drinks are an example of many alternatives to maintain human health, especially the digestive system. Bacterial isolates from sea cucumber, especially in the intestinal organs, has been proven capable to fight the growth of pathogenic bacteria. The ability of these isolates has more benefits that are useful as an inoculum in probiotic drinks. The purpose of this study was to determine the antibacterial and enzymatic potential of 3 types of intestinal bacteria of black sea cucumber (Rothia sp., Listeria sp., and Micrococcus sp.). This research was conducted in January-March 2019 with a preliminary test method in the form of an antibacterial test then followed by an enzymatic test (amylolitic, proteolytic, and cellulolytic). Quality test of probiotic drinks was also carried out and compared with the SNI standard. The result showed that the bacterial isolates from Rothia sp., Listeria sp., and Micrococcus sp. has the ability to inhibit and fight diarrhea-causing bacteria (Bacillus cereus). These isolates also capable to degrade protein and amylum, therefore produced SNI-approved probiotic drinks' quality. The average diameter of the inhibition zone in the preliminary tests was 19.15 mm, therefore classified as strong activity category. The results in the proteolytic test, showed the diameter of the inhibiton zone produced, ranged from 5,12 – 6,10 mm. Later on, the diameter of the inhibition zone on amylolitic test, ranged from 5,67 – 7,23 mm. Observation of the probiotic drinks' qualities containing isolates of Micrococcus sp. showed to have a slight difference with other samples due to the liquid's character is unhomogenous and tends to precipitate.

Index Term: Probiotics, *Rothia* sp., *Listeria* sp., *Micrococcus* sp., Yoghur

Introduction:

Sea cucumbers or holothurians (Holothuroidea, Echinoderms) are one of the specific and easily recognizable groups of marine biota. Sea cucumber's body shape in general is cylindrical, extending from the tip of the mouth to the anus (orally-aborally). The mouth is at the anterior end and the anus is at the posterior end. Sea cucumbers contain bioactive as an antibacterial ingredient. Extracts of sea cucumbers can damage the cell walls and cell membranes of pathogenic bacteria [1].

Bacteria are a class of organisms that do not have a nuclear envelope. Bacteria have genetic information in the form of DNA that is not specifically localized [2]. Bacteria can play a role in human life, such as preservatives and against pests and diseases [3].

Lactic acid bacteria (LAB) have amylolytic properties, namely lactic acid bacteria that can utilize starch as a substrate. Lactic acid bacteria also produce extracellular amylase. Lactic acid bacteria can ferment starch directly into lactic acid [4]. LAB is a bacterium that is classified as having a key role in the food world. The use of LAB in culinary is chosen. It has a function as a food-grade microorganism which is a microbe that does not threaten human health because it does not produce toxins. LAB was chosen because it contains microbes that are beneficial to the body [5].

This research is a follow-up study from Pringgenies et al. (2018)[6], using 3 isolates, namely Rothia sp., Listeria sp., Micrococcus sp. The three genera are symbionts of black sea cucumbers. The bacteria were carried out preliminary tests by looking for isolates that were active against the pathogenic bacteria Bacillus cereus and continued with enzymatic tests.

Methods:

This research was conducted on January-March 2019. Isolates Rothia sp., Listeria sp., and Micrococcus sp. was taken from the digestive symbiont of Black Sea Cucumber (Holothuria atra) in Bandengan Beach, Jepara. Exploratory method was used in this research. The explamatory method is a study of concept consolidation of preliminary research which can be used in a wider scope of research [7]. Prelinimary test was the initial step necessary to this research. The tests were implemented by observing the antibacterial activities in Rothia sp., Listeria sp., dan Micrococcus sp. against Bacillus cereus pathogen, using methods by Rahmiati & Mumpuni (2018)[8].

Enzymatic assay was conducted by seeing the proteolytic, amylolitic, and cellulolitic activities. The enzymatic assay was implemented using Marine Zobell agar medium, enriched with specific substances. The proteolytic assay was carried out to see the probiotic isolate candidate using enriched media with skim milk (1%). The amylolitic assay was carried out to see the amylolytic enzyme production using soluble starch (1%) enriched media. The cellulolitic assay was carried out to see the cellulolitic enzyme production using CMC (1%) enriched media [9].

Method of application of bacteria into probiotic beverages, was carried out with several production steps. The Total Lactic Acid test was condcted using Total Plate Count (TPC) method referring to Fardiaz (1993)[10]. The ash & water content were also analyzed using meathods referring to Wahyudi (2015)[11] and Masykur & Kusnadi (2015)[12].

Result:

The preliminary test results in this study were that Listeria sp. has a very strong antibacterial activity by showing an inhibition zone of 20 mm in diameter. While Rothia sp. and Micrococcus sp. have an inhibition zone smaller than 10 mm which indicates that their antibacterial activity is weak based on the diameter of the inhibition zone according to David & Stout (1971)[13]. These results prove that the gut bacteria of black sea cucumbers can inhibit bacterial growth. This is by research conducted by Sari & Ma'ruf (2014)[14].

Table 1: Preliminary Test Result

No.	Bacteria Isolate	Inhibit zone diameter (mm)
1.	*Listeria* sp.	12,5
2.	*Rothia* sp.	28
3.	*Micrococcus* sp.	22,7

Inhibit zone by Dvid and Stout (1971), D= ≥20 mm, very strong, D= 10-20 mm,strong, D= 5-10 mm medium, D= ≤5 mm, weak.

The results of the enzymatic test on Micrococcus sp. had the smallest proteolytic ability with an inhibition zone diameter of 5.12 cm, Listeria sp. of

5.75, and the smallest inhibition zone was Rothia sp. of 5.12 cm. The isolate that had the highest amylolytic activity was Rothia sp. with an inhibition zone of 7.23 cm, Micrococcus sp. of 6.33, and the isolate with the lowest inhibition zone was Listeria sp. of 5.67 cm. The results of the enzymatic assay on the three bacterial isolates showed that there was no cellulolytic ability at all. The inhibition zone formed was the result of hydrolysis of protein and starch substrates contained in marine zobell media by protease and amylase enzymes produced by bacterial isolates.

Table 2. Enzimatic Test Result

No	Bacteria Isolate	Degradation zone (cm)		
		Amilolitik	Proteolitik	Selulolitik
1.	*Listeria* sp.	5,67	5,75	-
2.	*Micrococcus* sp.	6,33	6,10	-
3.	*Rothia* sp.	7,23	5,12	-

The table of pH test results shows that all samples are in the pH range of 5-6. The results of the pH test were classified as normal probiotic milk products. This is because the pH value of the product with different isolates is close to the pH 4.5 set by the Food Standard Australia New Zealand (2014)[15].

Table 3. Ph Test Result

No	Bacteria Isolate	pH	
		0 Hour	18 Hour
1.	Susu murni	6	6
2.	*Rothia* sp.	6	5
3.	*Listeria* sp.	6	6
4.	*Micrococcus* sp.	6	5

The table of lactic acid test results shows that the total lactic acid bacteria is in the range of 11.2 x 10^7 CFU/ml - 31.3 x 10^7 CFU/ml. The value of lactic acid based on research results is still in accordance with SNI (2981:2009)[16], which is at least 10^7 CFU/ml. Total lactic acid bacteria that do not match the SNI standard (2981: 2009) will result in not optimally killing Bacillus cereus. This is confirmed by Setiarto et al. (2017)[17], that probiotics are live microorganisms in the form of lactic acid bacteria which if consumed in sufficient quantities will benefit the host.

Table 4. Lactic Acid Test Result

No	Bacteria Isolate	CFU/mL	SNI-Standart (2981:2009)
1.	Fresh Mik (10^{-6} & 10^{-8})	10,9 x 10^7	Min. 10^7 CFU/ml
		9,9 x 10^7	
2.	*Micrococcus* sp. (10^{-6} & 10^{-8})	11,2 x 10^7	
		8,6 x 10^7	
3.	*Listeria* sp. (10^{-6} & 10^{-8})	31,3 x 10^7	
		27,2 x 10^7	
4.	*Rothia* sp. (10^{-6} & 10^{-8})	28 x 10^7	
		31 x 10^7	

Discussion:

Lactic acid bacteria are microorganisms capable of fermenting starch directly into lactic acid. Lactic acid bacteria are the main requirement in the manufacture of probiotics because of their role as food grade microorganisms. These microbes will be inserted into the human body and are able to fight disease-causing microorganisms. Bacterial isolates from the intestines of sea cucumbers can be used as a source of lactic acid bacteria. Based on this, the purpose of this study was to determine the enzymatic activity of isolates of Rothia sp., Listeria sp., and Micrococcus sp. in the intestine of Holothuria atra as a source of lactic acid bacteria. This research was conducted in January – March 2019 with preliminary tests, enzymatic tests (amylolytic, proteolytic, and cellulolytic), and bacterial quality tests. The results showed that the isolates of Rothia sp., Listeria sp., and Micrococcus sp. able to fight the bacteria Bacillus cereus. The zone of inhibition generated in the preliminary test against bacteria Rothia sp., Listeria sp., and Micrococcus sp. respectively are 28 mm, 12.5 mm, and 22.7 mm. Based on the

results of amylolytic and proteolytic tests, isolates of Micrococcus sp. has the largest drop zone. The degradation zone in the cellulolytic test did not appear in the three isolates. Bacterial quality test showed that the concentration of lactic acid from the three isolates effectively inhibited the growth of Bacillus cereus bacteria.

Conclusion:

Intestinal symbionts of sea cucumbers Rothia sp., Listeria sp., and Micrococcus sp. has lactic acid levels ranging from 11.2 x 10^7 CFU/ml - 31.3 x 10^7 CFU/ml so it can kill Bacillus cereus. The bacterial isolate has the potential to be used as a probiotic drink.

Appendix An Appendix Title

Appendixes, if needed, is numbered by A, B, C... Use two spaces before APPENDIX TITLE.

Acknowledgment:

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