Exploration of extraceluller enzymes-producing bacteria symbiont Sponge *Phorbas* sp. from Nngge Island, Sibolga

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Abstract *This study aims to determine the bacteria that is in symbiont sponges and has the potential to produce extracellular enzymes such as protease, amylase, cellulase and lipase. The sea sponge obtained from Ngge Island, Sibolga is a type of Phorbas sp. The enzymatic activity test was carried out. Bacterial isolates obtained from the sponge Phorbas sp. there are 7 bacterial isolates. Seven bacterial isolates had the ability to produce extracellular enzymes and proteolytic, amylolytic, lipolytic and cellulolytic bacteria. Proteolytic activity using test media enriched with skim milk (1%), amylolytic activity using starch-enriched test media (1%), cellulolytic activity using CMC enriched test media (1%) and lipolytic activity using Tween 80 enriched test media (1%). Based on the results of the research, seven isolates of sponge symbiont bacteria have enzymatic activity of amylase and cellulase, namely bacterial isolates P1, P2, P3, P4, P5, P6, P7 and six (6) bacterial isolates which have protease and lipase enzymatic activity are bacterial isolates P1, P2, P3, P4, P6, P7.* [EXPLORATION OF EXTRACELULLER ENZYMES-PRODUCING BACTERIA SYMBIONT SPONGE *PHORBAS* SP. FROM NNGGE ISLAND, SIBOLGA] ***(J. Math. Nat. Sci., 1(1): 30 - 34, 2021)***

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Introduction

Enzymes are part of proteins that act as catalysts for chemical reactions and enzymes also have specificity for the reactions that are catalyzed and the molecules that become their substrates (Okoko and Ogbomo, 2010).

The use of enzymes in the industrial sector in Indonesia has recently increased sharply. According to the Agency for the Assessment and Application of Technology (2015), 99% of the need for enzymes or biocatalysts for industry is still imported from other countries. Meanwhile, the use of enzymes in the industrial sector in Indonesia reached 2500 tons with an import value of 187.5 billion in 2015. Therefore, efforts are needed to produce enzymes in Indonesia.

Indonesia is the largest archipelagic country in the world, which also has a large wealth of marine natural resources. One of the efforts that need to be done to overcome the increased use of enzymes in Indonesia is to use bioactive compounds produced by several marine organisms, namely sponges.

Sponges are one of the biota components that make up coral reefs that have bioactive potential which has not been widely utilized. These marine animals contain active compounds whose percentage of activity is greater than those produced by land plants (Muniarsih and Rasyid, 2010). Bioactive compounds produced by sponges by utilizing the enzymatic activity of bacteria in symbiosis with sponges. According to Feby and Nair (2010), bacteria associated with sponges are an excellent source of extracellular hydrophilic enzymes because the surface and internal space of the sponge are richer in nutrients.

In the study of Gultom et al. (2017), 17 bacterial isolates were obtained from the two sponges consisting of 8 bacterial isolates from Haliclona sp.2 and 9 bacterial isolates from Axinellid sp. According to Marzuki et al (2014), bacteria that are in symbiosis with sponges of the species

Callyspongia sp. the Callyspongiidae family produces a group of gram-negative bacteria and produces the enzyme amylase. The bacterial isolate with ID 19TR identified as Sphinogomonas phyllosphaerae strain FA2 was able to break down protein and had the highest protease enzyme activity (Riffiani and Sulistinah, 2010).

Therefore, the use of sponge symbiont bacteria is needed to produce enzymes needed in various fields, especially the food industry and aquaculture. Based on the above studies, it is necessary to conduct research on the screening and identification of bacteria in symbiosis with sponges as producers of extracellular enzymes such as proteases, amylases, cellulases and lipases.

Materials and Methods

Location and time of research. The sampling of sponges was carried out purposively, namely by tracing the seabed by scuba diving at a sea depth of ± 5 - 10 m in the Pulau Ngge of Sibolga, North Sumatera. The sponge is taken and cut using a knife and then put in the sample bag. The sponge is then washed with sea water and put in a sample bag that has been filled with sea water and given oxygen, then stored in a cool box. Then taken to the laboratory for identification.

Isolation and Purification of Spongy Symbiont Bacteria. The sponge sample was washed then cut into small pieces and weighed 1 gram then crushed using a mortar. After that, it is put into a beaker and then dissolved in 9 mL of sterile sea water as a stock solution. Do a 10-1 to 10-4 dilution. 1 mL of sample was taken from a 10-1 - 10-4 dilution and inoculated into each petri dish filled with 20 mL of MA media aseptically using the pouring method, then incubated at 37oC for 1-2 days. After incubation, observation of colony cell morphology was carried out, namely shape, color, elevation and edge. From the bacterial culture, 1 ose was taken aseptically and then inoculated by scratching it on MA media, then incubated at 37oC for 24 hours (Figure 1) (Marzuki et al., 2014; Setyati and Subagiyo, 2012; Restuati and Gultom, 2012).

Enzymatic Activity Test of Spongy Symbionts Bacteria. Each pure isolate was inoculated into 5 mL of MB media in a different test tube, then homogenized using a vortex. Isolates were incubated at room temperature for 24 hours.

a. Protease Enzyme Activity Test

The test medium used was MA media enriched with skim milk (1%). Sterile disc paper is placed on the test media. Each liquid culture was homogenized and then inoculated on sterile disc paper. Subsequently incubated at room temperature for 24 hours. The identification of protease enzyme activity was indicated by a clear zone formed around the disc paper on a white background (Figure 2a) (Setyati and Subagiyo, 2012).

b. Amylase Enzyme Activity Test

The test medium used was MA media which had been enriched with starch (1%). Sterile disc paper is placed on the test media. Each liquid culture was homogenized and then inoculated on sterile disc paper. Subsequently incubated at room temperature for 24 hours. Identification of amylase enzyme activity was carried out by pouring Gram iodine solution onto the culture media as indicated by the formation of a clear zone around the disc paper with a dark blue background (Figure 2b) (Setyati and Subagiyo, 2012).

c. Cellulase Enzyme Activity Test

The test medium used was MA media that had been enriched with CMC (1%). Sterile disc paper is placed on the test media. Each liquid culture was homogenized and then inoculated onto sterile disc paper. Subsequently incubated at room temperature for 48 hours. Identification of cellulase enzyme activity was carried out by pouring a solution of Gram iodine onto the culture media, which showed the formation of a clear zone around the disc paper with a pink background (Figure 2c) (Setyati and Subagiyo, 2012).

d. Lipase Enzyme Activity Test

The test medium used was MA media that had been enriched with Tween 80 (1%). Sterile disc paper is placed on the test media. Each liquid culture was homogenized and then inoculated onto sterile disc paper. Subsequently incubated at room temperature for 24 hours. After incubation, the activity of the lipase enzyme is indicated by the formation of cloudy white fatty acid deposits that form around the disc paper (Figure 2d) (Setyati and Subagiyo, 2012).

Results

The sponge is obtained from shallow waters of Sibolga with a distance of about ± 500 m from the edge of Pandan Beach and with a depth of about ± 5 - 7 m by scuba diving. The sponge obtained has red characteristics, has a brittle or soft texture, has a thin layer and leaves a red tuft when touched. The results from the isolation of the sponge Phorbas sp. As many as 7 bacterial isolates were identified macroscopically (Table 1) and microscopic identification (Table 2).

Seven bacterial isolates tested were obtained 6 bacterial isolates that produced protease and lipase enzymes and 7 bacterial isolates that produced amylase cellulase enzymes. can be seen in Table 3 and Figure 3.

Figure 1. Isolate of bacteria symbionts *Phorbas* sp.

Table 1. Identification table of Phorbas sponges symbiont bacteria isolates macroscopically.

Isolate	Colony Form	Color	Elevation	Edge of the Colony
P1	Irregular	Red	Flat	Lobate
P2	Circullar	Yellow	Convex	Entire
P3	Irregular	White	Flat	Lobate
P4	Irregular	White	Flat	Undulate
P5	Irregular	Gray	Flat	Curled
P6	Circular	Red	Flat	Lobate
P7	Circular	Red	Flat	Entire

Table 2. Identification microscopically bacteria symbionts *Phorbas* sp.

Isolate	Form	Gram
P1	Coccus	Negative
P2	Coccus	Positive
P3	Coccus	Positive
P4	Basil	Positive
P5	Coccus	Positive
P6	Coccus	Negative
P7	Basil	Negative

Discussion

The protease enzyme production activity test can be seen from Table 3. Qualitatively, the ability of bacterial isolates to produce protease enzymes is a reflection of the hydrolysis activity to break down proteins seen from the large clear zone produced around disc paper. Bacteria that are able to secrete protease enzymes have the ability to hydrolyze protein compounds into oligopeptides, short-chain peptides, and amino acids (Setyati and Subagyo, 2012). In the research of Shanmughapriya et al. (2008), the activity of protease enzyme production was highest from the bacteria symbiont spons Fasciospongia cavernosa taken from the Peninsula of the east coast of India.

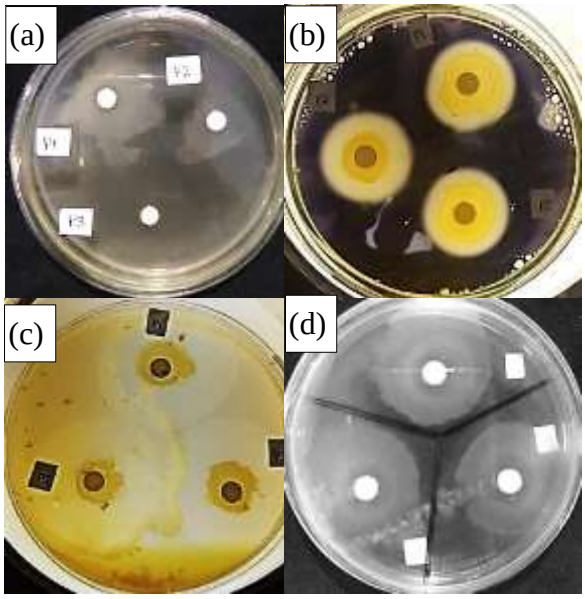

Figure 2. Results of Enzymatic Activity of Spongy Symbionts Bacteria. (A) Protease enzyme activity test; (B) Test the amylase enzyme activity; (C) Cellulase enzyme activity test; (D) Lipase enzyme activity test.

The amylase enzyme produced by bacterial isolates is characterized by the for mation of a clear zone on the uij medium containing starch with the addition of iodine solution. The clear zone that is formed around the disc paper on the agar media is dark blue in color, the bacterial isolate is able to hydrolyze the starch content contained in the media while the dark blue media contains the starch content in the media which has not been hydrolyzed. The addition of a gram iodine solution serves to determine the ability of bacteria to use starch. The degradation that occurs in starch is known by the loss of iodine-colored material (Marzuki et al., 2014).

Amylase can hydrolyze complex sugars such as starch into simple sugars such as glucose, maltose and dextrins (Setyati et al., 2016). Bacteria that have cellulolytic activity have the ability to

produce cellulase enzymes that are secreted into their environment. Cellulolytic enzymes work to hydrolyze cellulose into simpler polysaccharides called cellodextrin (Zhang and Kim, 2010). The resulting cellulase enzyme is an inductive enzyme whose biosynthesis is influenced by an inducer, namely cellulose found in the CMC substrate. Cellulase will work in the process of breaking down cellulose into glucose (Moat et al., 2002). This enzyme works to catalyze the breakdown of fats and oils which are then released into free fatty acids, diacyglycerols, monoglycerols and glycerol (Zhang and Kim, 2010).

Table 3. Table of the results of enzymatic activity of the bacterial symbionts *Phorbas* sp.

Isolate	Hydrolytic Zone Diameter (mm)			
	Protease	Amylase	Cellulases	Lipase
P1	1.72±0.29	20.50±0.67	29.31±0.82	19.0±0.33
P2	1.62±1.08	9.50±5.67	25.10±1.40	24.33±2.55
P3	2.47±0.36	24.16±2.55	31.42±1.71	29.34±1.69
P4	1.35±0.27	23.66±0.55	29.56±2.31	1.93±0.27
P5	-	24.33±2.56	29.66±1.11	-
P6	2.27±0.26	24.83±7.11	33.71±1.85	15.36±2.38
P7	0.67±0.45	25.0±5.33	13.05±5.95	3.51±0.62

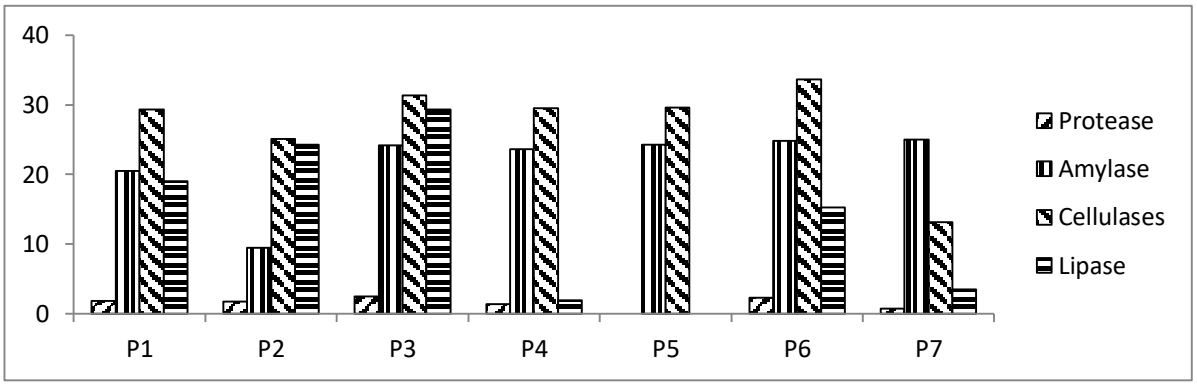

Figure 3. Graph of the Enzymatic Activity of bacteria symbiont *Phorbas* sp.

Conclusion

From this study it can be concluded that there are seven types of extracellular enzyme-producing bacterial symbionts sponge. There were isolates namely PI, P2, P3, P4, P6 and P7 a bacterium can hydrolysis of protein, lipid, amylum and cellulose; isolate namely P5 can hydrolysis of amylum and cellulose.

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