p-ISSN: 2442-9481
e-ISSN: 2685-7332

Isolation and Selection of Extracellular Enzymes in Sponge Symbiont Bacteria (Porifera: Demospongiae) from Tablolong Beach

Lintang A. M. Dima[1], Andriani Rafael[1], Sonya, T. M. Nge[1](*), Ocky K. Radjasa[2], Tiodor S. J. Manalu[3], James Ngginak[1]

[1] Department of Biology Education Study Program, Artha Wacana Christian University,
Jl. Adi Sucipto No.147, Oesapa, Klp. Lima District, Kupang City, East Nusa Tenggara
[2] Indonesian Institute of Sciences, Jl. Jend. Gatot Subroto 10, Jakarta 12710
[3] Department of Biology Teacher, Del Senior Highschool North Sumatera
Jl. YP. Arjuna, Desa Sitoluama, Kec. Laguboti, Sitoluama, Toba,
North Sumatera 22381

*__Corresponding author__: sonyatitin@gmail.com

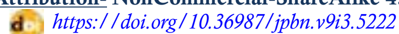
Submitted September 16th 2023 and Accepted November 04th 2023

Abstract

Marine biota has many benefits for human life. Sponges are a species of marine biota that can be used as a producer of antimicrobial compounds. The bacteria found in sponges have an important role in the continuity of life in the sea. The symbiotic lifestyle that occurs in bacteria and sponges has the opportunity to form substitutions for the content of secondary metabolites and enzymes, especially extracellular enzymes (amylase, protease, cellulose and lipase). This study aims to determine how to isolate sponge symbiotic bacteria and identify spongy symbiotic bacteria. The method used is purposive to take sponges. Characterization of bacteria was carried out based on morphology and gram staining. Enzymatic bacterial selection was carried out by testing the activity of the amylase enzyme (soluble starch), lipase enzyme (Teen 80), protease enzyme (skim milk) and cellulose enzyme (carboxyl methyl cellulose). Data analysis was performed in a qualitative descriptive manner by measuring the clear zone in the extracellular enzyme test results. Meanwhile, other research parameters measured in this study were temperature, salinity and pH. The results showed that out of 47 isolated bacteria and 33 of them had extracellular enzymes with 10 bacteria had amylase enzymes, 27 bacteria had lipase enzymes, 2 bacteria had protease enzymes and 1 isolate had cellulose enzymes

Keywords: *Bacteria, Extra-cellular enzymes and Sea sponges*

Jurnal Pembelajaran dan Biologi Nukleus (JPBN) by LPPM Universitas Labuhanbatu is under a Creative Commons Attribution- NonCommercial-ShareAlike 4.0 International License (CC BY - NC - SA 4.0)


https://doi.org/10.36987/jpbn.v9i3.5222

INTRODUCTION

Indonesia is a country renowned for its substantial water area, which sustains its marine biodiversity, which is exceptionally abundant. Seaweed, fish, crustaceans, seagrass meadows, mangroves, and various species of microorganisms comprise Indonesia's maritime potential (Damar et al., 2021). The marine biodiversity of Indonesia, according to (Rambu et al., 2021), has the potential to advance the pharmaceutical, agricultural, and health industries. East Nusa Tenggara is a geographical area characterized by abundant and diverse marine resources (Takeltanu et al., 2018)

Sponge biota have the potential to contribute significantly to human existence. Upon reaching maturity, sponges lack structured tissues and maintain a sedentary lifestyle. Although sponges inhabit both freshwater and saltwater, the majority of them maintain permanent residence in the sea, ranging from modest coastal waters to depths of 1000 meters. Sponge life is characterized by its attachment to marine substrates, including sand, pebbles, and decomposing coral. Some sponges resemble fingers, floral containers, monuments, tubes, drillers, vines, massives, spheres, semi-balls, and fingers, among other forms. The physical, chemical, and biological surroundings significantly impact the external morphology of the sponge (Hutasoit et al., 2017). Sponges have been found to have utility across a multitude of domains in human existence, being employed as antimicrobial, antitumor, antiviral, antibacterial, and antimalarial medications (Pasodung et al., 2018). Spoons can be used in many areas because they contain nitrogen, peptide compounds, terpenoids, steroids, acetogenins, alkaloids, and cyclic halides (Cita et al., 2015). It is noteworthy to mention that sponges engage in a symbiotic life cycle with numerous organisms, including bacteria.

According (Remijawa et al., 2020) conducted research in the waters of Tablolong and reported the discovery of bacteria that produce cellulose enzymes. Sponge-symbiont microorganisms also contain the lipase enzyme (Hutasoit et al., 2017). Amylase, an enzyme present in sponge symbiont microorganisms, is capable of decomposing starch into basic compounds (Murtius et al., 2017). Microorganisms, plants, and vertebrates are the sources of amylase enzymes. Andoamylase and exoamylase are the two subtypes of amylase (Arfah et al., 2014). Protease is an enzyme responsible for the hydrolysis of proteins into amino acids. Microorganisms and vegetation are the sources of this enzyme. Protease is a type of hydrolase that can break down proteins into basic molecules like amino acids and short oligopeptides (Siallagan et al., 2020). Tablolong Beach is one of the tourist attractions situated in the Kupang Regency, West Kupang District. Tablolong Beach is located approximately 30 kilometers from the city of Kupang. The presence of biodiversity and exceptionally pure waters at this shore make it a possible indicator of environmental health (Belang et al., 2018).

METHOD

Research time and location

Sampling was carried out at Tablolong Beach, West Kupang Regency. Testing of Sponge Samples was carried out at the Microbiology Laboratory of Artha Wacana Christian University, Kupang.

Picture 1. Map of the research location for Tablolong Beach, NTT

Research instruments and materials

Snorkeling equipment was utilized to survey and search for sponges in the sea, scissors were employed to cut sponge specimens to a length of 5-10 cm, sample bottles were utilized to store collected specimens, refractometers were employed to measure the salinity of sea water, motorboats served as means of transportation to the sea, a cool box is employed to temporarily store specimens at sea, an underwater camera was employed to document activities and specimens obtained while at sea, and stationery was employed to record and document activities carried out at sea.

Testing equipment in the laboratory "Petri disc" was used as a place to culture and test isolates, a needle was used to collect bacterial isolates, a bunsen was used to reduce contamination during culture, an Erlenmeyer was used to make media, a test tube was used as a container for tilting media, analytical scales were used to measuring the materials used in making the media, a spreader tool was used to spread the liquid isolate on the media, tweezers was used to take the specimen, a scalpel was used to cut the specimen, an autoclave was used as a sterilization tool, a hot plate was used to heat and homogenize the media, a vortex was used to homogenize the isolate or media, a shaker was used to help culture isolates with a shaking movement, an incubator was used to grow isolates at a measured temperature, a microscope was used to observe morphological characters with a certain magnification, mortal and pastel were used to smooth the specimen, a caliper was used to measure the area of the clear zone, a micropipette and tips were used as tools to take materials or solutions of a certain size (0.5 µl - 1 ml), glass beakers were used as containers for making media, tube racks were used to store test tubes and personal protective equipment (coats, masks, gloves) was used when carrying out laboratory activities (Setyati & Subagiyo, 2012).

p-ISSN: 2442-9481
e-ISSN: 2685-7332

The materials used in this research were Zobell Broth enriched with 1% pure agar as a medium for growing bacteria in solid form, Natrium Broth as a medium for growing bacteria in liquid form, distilled water as a solvent, soluble strach (1%) and gram iodine solution as ingredients. amylase production activity test, skim milk (1%) as a test material for protease production activity, tween 80 as a test medium for lipase production activity, CMC (1%), gram iodine solution as a test medium for cellulase production activity and paper discs as a place for pure culture. will be tested as well as gram reagents for gram testing on bacteria (Setyati & Subagiyo, 2012).

Research design

This research employed a qualitative descriptive method to look at the extracellular enzyme content found in bacteria that form symbionts with sponges.

Research procedures

1. Preparation phase
   a) Field survey to observe and determine sampling locations.
   b) Prepare tools and materials used in the research.
2. Sampling phase

Sampling was carried out purposively, namely by tracing the seabed. The sponges were collected by snorkeling at Tablolong Beach, West Kupang at a depth of 1-2 meters. Next, the sponge samples are cut and put into a Coolbox containing ice packs for further analysis in the laboratory (isolation stage). Once in the laboratory, the sample was measured with a ruler, then photographed, then crushed and taken as needed at the dilution stage.

Cultivation of Sponge Symbiont Bacteria

Sponge samples were crushed using mortar and pastel aseptically. After crushing, take 1 gram of the sponge, put it in a test tube containing 9 ml of sterile seawater (10^{-1}), then shake it until it is homogeneous. Next, 1 ml of the 10^{-1} dilution was taken with a micropipette and then placed in 9 ml of sterile sea water and a 10^{-2} dilution was obtained. Dilution was carried out until a dilution of 10^{-7} was obtained. At each dilution, 100 μl of the bacterial suspension was taken using a micropipette and inoculated into a petri dish filled with Zobell broth media. Next, flatten using a spreader and incubate for 2x24 hours at 37°C.

Characterization of Bacterial Isolates

The bacterial isolate was subjected to characterization via the gram staining method. Alcohol was used to clear the glass object that had been roasted over a flame. Then, it was transferred into one dose of the bacterial culture suspension to a glass surface and pass it over a Bunsen flame in an aseptic manner. Then, it was drizzled with crystal violet, distribute it evenly, and allow it to settle for one minute before rinsing it under running water and drying it. Then, it was allowed to remain for one minute while drenched in iodine; clean under running water and dry. Following that, it was rinsed for 30 seconds with ethanol bleach or decolorizer, followed by running water and drying. After soaking for one minute in Safranin-treated water, it was rinsed under running water

and allow it to dry. After observing the bacteria under a 100-400x magnification, the gram type of the bacteria was determined. Gram-negative bacteria were colored red, whereas gram-positive bacteria are colored purple (Remijawa et al., 2020).

Separation and Purification of Isolates

Separation and purification of bacterial isolates was carried out using the streak method. In each petri dish, at each dilution, colonies of bacteria were taken that showed different morphology and color. Next, each colored colony was streaked on the surface of the Zobell medium in each prepared petri dish. Next, the petri dishes were incubated at room temperature for 2x24 hours and the growth was observed, whether it had separated and become a pure culture or not. If there was still a mixture of bacteria in the petri dish, then it was separated until a pure culture was obtained.

Isolation and Purification of Sponge Symbiont Bacteria

The growing bacterial colonies were observed for their morphology and colony color. Each different morphological and color appearance in each petri dish was analyzed. The streak method was used to separate and purify each bacteria on the surface of the Zobell medium. The petri dish was incubated for 2x24 hours and the growth of the bacteria that had been grown was observed. If a pure culture had not been obtained, then the separation was carried out again using the break method to obtain a pure culture (Setyati & Subagiyo, 2012). After obtaining the pure culture, the bacterial culture was stored in a test tube containing slanted Zobell media using the streak method. The results were isolated on slanted Zobell media and then incubated for 24 hours in an incubator.

Sample Analysis

This enzymatic bacterial selection was carried out based on a modified procedure. Each slant media isolate was taken using a loop needle and then inoculated into 5 ml of liquid marine Zobell media in a different test tube. The isolate was incubated on a shaker at room temperature for 24 hours.

Protease Enzyme Production Activity Test

The test medium used was Zobell enriched with skim milk (1%). Sterile paper disks are placed on top of the skim milk agar. Each liquid culture was inoculated onto a paper disc, then incubated at room temperature for 24 hours. Identification of proteolytic activity was carried out by measuring the clear zone formed around the paper disc using a caliper (Setyati & Subagiyo, 2012).

Amylase Enzyme Production Activity Test

The test medium used was Zobell media which had been enriched with soluble strach (1%). A sterile paper disc was placed on top of the test medium. Each liquid culture was inoculated onto a paper disc, then incubated at room temperature for 24 hours. Identification of amylolytic activity was carried out by pouring Gram Iodine onto the culture medium. The clear zone formed around the paper disc was measured using a caliper (Setyati & Subagiyo, 2012).

p-ISSN: 2442-9481
e-ISSN: 2685-7332

Cellulase Enzyme Production Activity Test

The test medium used was Zobell's medium enriched with CMC (1%). A sterile paper disc was placed on top of the test medium. Each liquid culture was inoculated onto a paper disc, then incubated at room temperature for 48 hours. Identification of cellulotic activity was carried out by pouring Gram Iodine solution onto the culture medium. The clear zone formed around the paper disc was measured using a caliper (Setyati & Subagiyo, 2012).

Lipase Enzyme Production Activity Test

The test medium used was Zobell medium enriched with Tween 80 (1%). A sterile paper disc was placed on top of the test medium. Each liquid culture was inoculated onto a paper disc, then incubated at room temperature for 24 hours. After incubation, lipolytic activity was indicated by a cloudy white zone that formed around the paper disc. The clear zone formed was then measured using a caliper (Setyati & Subagiyo, 2012).

Data analysis

Data on isolates of sponge symbiotic bacteria that produce extracellular enzymes were analyzed descriptively qualitatively, namely by searching and compiling data systematically according to experimental methods. The formation of a clear zone in the results of the extracellular enzyme test was the point of analysis (Setyati & Subagiyo, 2012).

RESULTS AND DISCUSSION

Environmental Parameters

The sponge samples used were taken from Tablolong beach at a depth of approximately 5 meters. Before sampling is carried out, environmental parameters are first measured to see the quality of Tablolong waters. Water quality regarding the presence of organisms and microorganisms such as bacteria in these waters. The environmental parameters measured are temperature, salinity and pH. The results of measuring environmental parameters can be seen in table 1.

Table.1 Environmental factor

Temperature (°C)	Salinity (°/∞)	Acidity (pH)
23	39	8

Identifying sponges and sponge symbiont bacteria

After measuring environmental parameters, sponge identification was carried out based on guidelines, namely by comparing the morphology of the sponge (Table 2.).

Table 2. Identification of sponge symbiont bacteria

Sample	No	Isolate Code	Morphology				Gram
			Color	Surface	Edge	Cell shape	
Xestospongia sp. sponge	1	TB1-01-1.1	White	Convex	Flat	rod-shaped	+
	2	TB1-01-1.2	Yellow	Flat	Wavy	rod-shaped	+
	3	TB1-01-1.3	White	Flat	Wavy	rod-shaped	+
	4	TBI-01-3.1	White	Flat	Irregular	rod-shaped	+
	5	TBI-01-3.2	White	Convex	Flat	rod-shaped	+
	6	TBI-01-4.1	White	Flat	Branching	rod-shaped	+
	7	TB1-01-4.3	White	Flat	Branching	rod-shaped	-
	8	TB1-01-4.4	White	Flat	Wavy	Round	+
	9	TB1-01-5.1	Yellow	Flat	Branching	rod-shaped	+
	10	TB1-01-5.2	White	Flat	Wavy	rod-shaped	-
	11	TB1-01-6.1	Yellow	Flat	Branching	rod-shaped	-
	12	TB1-01-7.1	White	Flat	Wavy	rod-shaped	+
	13	TB1-01-7.2	White	Flat	Branching	rod-shaped	+
	14	TB2-01-2.1	White	Flat	Branching	rod-shaped	+
	15	TB2-01-2.2	White	Flat	Branching	rod-shaped	-
	16	TB2-01-2.3	White	Flat	Branching	Round	+
	17	TB2-01-2.4	White	Flat	Wavy	rod-shaped	-
	18	TB2-01-3.1	White	Convex	Branching	rod-shaped	+
	19	TB2-01-3.2	White	Flat	Branching	rod-shaped	-
	20	TB2-01-3.3	Cream	Flat	Branching	rod-shaped	+
	21	TB2-01-4.1	White	Convex	Wavy	rod-shaped	+
	22	TB2-01-5.1	White	Flat	Wavy	rod-shaped	+
	23	TB2-01-5.2	Yellow	Convex	Wavy	rod-shaped	+
	24	TB2-01-5.3	White	Flat	Wavy	rod-shaped	+
	25	TB2-01-6.1	White	Flat	Branching	rod-shaped	-
	26	TB2-01-7.1	White	Flat	Branching	Round	-
	27	TB2-01-7.2	Yellow	Flat	Branching	rod-shaped	-
	28	TB2-01-7.3	Yellow	Flat	Wavy	rod-shaped	+

p-ISSN: 2442-9481
e-ISSN: 2685-7332

***Acanthella sp.* sponge**	29	TB1-02-3.1	White	Flat	Flat	rod-shaped	+
	30	TB1-02-3.2	White	Convex	Wavy	Round	+
	31	TB1-02-4.1	White	Flat	Branching	rod-shaped	-
	32	TB1-02-5.1	Yellow	Flat	Branching	rod-shaped	-
	33	TB1-02-5.2	White	Convex	Branching	rod-shaped	+
	34	TB1-02-6.1	White	Flat	Branching	rod-shaped	-
	35	TB1-02-7.1	Orange	Convex	Flat	Round	+
	36	TB2-02-2.1	White	Convex	Branching	rod-shaped	+
	37	TB2-02-2.2	White	Flat	Branching	rod-shaped	+
	38	TB2-02-3.1	White	Flat	Branching	rod-shaped	-
	39	TB2-02-3.2	White	Convex	Branching	rod-shaped	-
	40	TB2-02-3.3	White	Flat	Flat	Round	+
	41	TB2-02-4.1	White	Flat	Branching	rod-shaped	+
	42	TB2-02-4.3	White	Flat	Branching	rod-shaped	-
	43	TB2-02-5.1	Yellow	Convex	Irregular	rod-shaped	+
	44	TB2-02-6.1	White	Flat	Branching	rod-shaped	-
	45	TB2-02-7.1	White	Flat	Wavy	rod-shaped	+
	46	TB2-02-7.2	Cream	Flat	Branching	rod-shaped	+
	47	TB2-02-7.3	White	Convex	Wavy	rod-shaped	-

Extracellular Enzyme Activity Test

The bacteria that have been identified are then tested for extracellular enzymes using sodium agar media enriched with skim milk (1%) for the protease enzyme test, soluble strach (1%) for the amylase enzyme test, Carboxy Methyl Celulase (1%) for the cellulase enzyme test and tween 80 for the lipase enzyme test. The results of the extracellular enzyme activity test can be seen in the figure 2.

DISCUSSION

Environmental Parameters

The data indicates that the temperature at the site of sampling is 23 degrees Celsius. The aforementioned temperature is classified within the mesophyll group, which is favorable for bacterial proliferation. Sponge survival generally requires a temperature of 29 degrees Celsius (Haedar. et al., 2016). However, the temperature in the waters of Tablolong was found to be lower than this threshold in this study, suggesting that sponges have the ability to acclimate and proliferate at varying temperatures. Bacteria exhibit a temperature range of 2 degrees Celsius, spanning from polar seawater to tropical seawater at 45 degrees Celsius.

p-ISSN: 2442-9481
e-ISSN: 2685-7332

Figure 2. Sponge (Sample) *Xetospongia sp*, *Acanthella sp.*

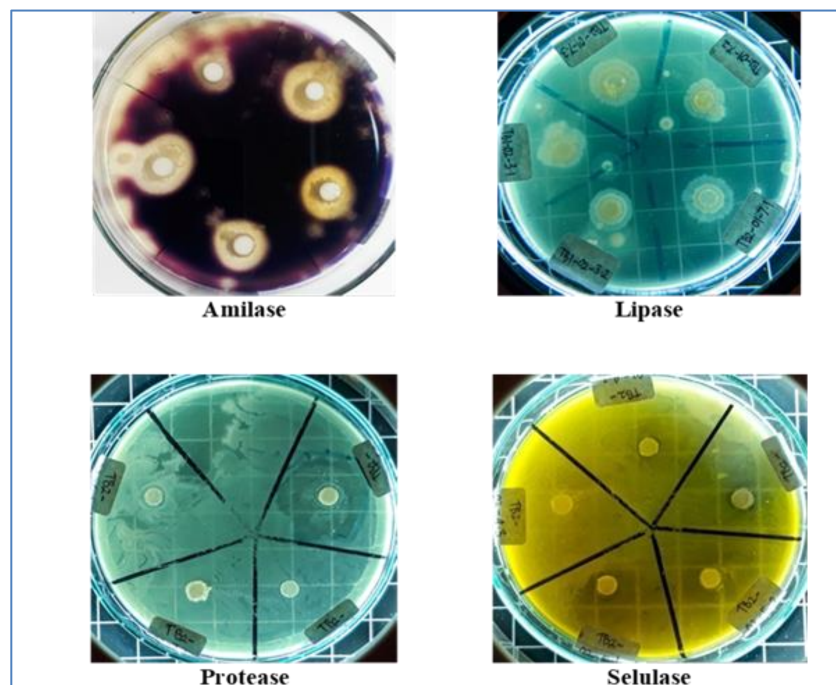


Figure 3. Amylase, Lipase, Protease, Cellulase enzyme activity test
Source: Research results

Table 3. Hydrolytic Zone Diameter

No	Isolate code	Hydrolytic Zone Diameter (mm)			
		Amylase	**Lipase**	**Protease**	**Cellulase**
1	TB1-01-1.1	0	10 ± 7,5	0	0
2	TB1-01-1.2	0	20± 0	0	0
3	TB1-01-1.3	0	14 ± 0	0	0
4	TBI-01-3.1	0	6 ± 2,5	0	0
5	TBI-01-3.2	0	8 ± 2	0	0
6	TBI-01-4.1	0	8 ± 1,5	0	0
7	TB1-01-4.2	0	0	0	0
8	TB1-01-4.3	0	8 ± 2	0	0
9	TB1-01-5.1	0	8 ± 4	0	0
10	TB1-01-5.2	0	10 ± 6	0	0
11	TB1-01-6.1	0	8 ± 6	6 ± 0,5	0
12	TB1-01-7.1	0	8 ± 6	0	0
13	TB1-01-7.2	30 ± 4	0	0	0
14	TB2-01-2.1	20 ± 3,5	0	0	0
15	TB2-01-2.2	16 ± 0	0	0	0
16	TB2-01-2.3	0	0	0	0
17	TB2-01-2.4	0	0	0	0
18	TB2-01-3.1	0	0	0	0
19	TB2-01-3.2	0	8 ± 5	0	0
20	TB2-01-3.3	0	8 ± 4	0	0
21	TB2-01-4.1	0	4 ± 3	0	0
22	TB2-01-5.1	0	0	0	0
23	TB2-01-5.2	0	0	0	0
24	TB2-01-5.3	0	0	0	0
25	TB2-01-6.1	0	0	0	0
26	TB2-01-7.1	0	12 ± 4	0	0
27	TB2-01-7.2	0	8 ± 2	0	0
28	TB2-01-7.3	0	12 ± 5	0	0
29	TB1-02-3.1	0	6 ± 2	0	0
30	TB1-02-3.2	0	10 ± 5	0	0
31	TB1-02-4.1	0	0	0	0
32	TB1-02-5.1	0	0	0	0
33	TB1-02-5.2	0	0	0	0
34	TB1-02-6.1	26 ± 2,5	0	0	0
35	TB1-02-7.1	26 ± 0	0	0	0
36	TB2-02-2.1	34 ± 5	8 ± 5	0	0
37	TB2-02-2.2	0	4 ± 0	0	0
38	TB2-02-3.1	0	0	0	0
39	TB2-02-3.2	0	0	0	0
40	TB2-02-3.3	0	0	0	0
41	TB2-02-4.1	0	0	0	4 ± 0
42	TB2-02-4.3	0	4 ± 1,5	0	0
43	TB2-02-5.1	0	4 ± 1	0	0
44	TB2-02-6.1	12 ± 2	8 ± 5	0	0
45	TB2-02-7.1	36 ± 3	10 ± 4	0	0
46	TB2-02-7.2	14 ± 1,5	8 ± 4	0	0
47	TB2-02-7.3	20 ± 7	6 ± 4	20 ± 0	0

At the sampling location, the salinity measurement was 39 o/∞. According to the results of these measurements, the salinity of the waters in Tablolong is quite high; this,

of course, inhibits bacterial proliferation. In general, Indonesian waters exhibit a salinity gradient of 30–35 o/∞ (Remijawa et al., 2020). (Patty, 2013) posits that salinitis susceptibility can be affected by precipitation, evaporation, ocean currents, and the volume of incoming freshwater. There are 29 salinias that are conducive to the growth of sponge biota (Haedar. et al., 2016). Nevertheless, the salinity level of the Tablolong waters was quite high during this investigation, suggesting that sponges can endure varying degrees of salinity.

The pH value at the sampling location is 8. Sponges have a pH value of 7, but some types of sponges can certainly adapt to environmental conditions. According to (Marzuki et al., 2018), sponge symbiont bacteria can live in a pH range of 4 to 9, but some bacteria can live in acidic or alkaline conditions. The results of these measurements show that bacteria can grow well at this pH value, namely 8.

Identify sponges and sponge symbiont bacteria

Table 2 presents the morphological characteristics of the identified isolates, which included bacteria with round and rod-shaped bacterial cells, flat and convex bacterial surfaces, and flat, curved, and irregular bacterial margins. The bacteria were yellow, white, and cream in color. The process of identifying bacterial isolates in accordance with Bergey's Manual of Bacteriology yielded sixteen bacterial isolates that were classified as belonging to the genus Bacillus. These isolates exhibited distinct characteristics, including a color range from white to yellow, a flat surface, uneven edges, and rod-shaped cells.

Additionally, thirteen bacterial isolates were identified as belonging to the genus Pseudomonas. These isolates were distinguished by their convex bacterial surfaces, rod- or round-shaped smooth bacterial margins, and white to yellow bacterial coloration. A total of nine isolates were classified as bacteria belonging to the genus Corynebacterium, characterized by their stem cell shape, whitish coloration, and convex bacterial surfaces with branched borders. A total of five bacterial isolates were classified under the genus Micrococcus. These isolates exhibited characteristics such as a round cell shape, a uniform bacterial surface, and undulating bacterial margins. Two bacterial isolates were identified as belonging to the genus Pseudovibrio; they possessed characteristics such as a yellow coloration, a convex surface, irregular borders, and a stem cell shape.

Identification of bacteria can be seen from the results of gram staining, which is an important technique for distinguishing gram-positive and gram-negative bacteria. The gram staining results showed that 30 bacterial isolates were gram positive. Bacteria that produce gram positive can be seen from the purple color resulting from gram staining. This purple color is caused by gram positive bacteria being able to retain the main dye crystal violet even though it has been washed with a decolorizer bleach solution. This is because gram-positive bacteria have a cell wall structure with a thick peptidoglycan content so that they can still bind the main dye and are not damaged when washed with a decolorizer. Apart from that, the cell walls of gram-positive bacteria contain teichoic acid and lipoteichoic acid (Sabdaningsih et al., 2013). Bacteria that are classified as gram positive are bacteria from the genus Bacillus, Micrococcus and *Corynebacterium*.

A total of seventeen gram negative bacterial isolates were identified. Gram-negative bacteria are identifiable by the red hue of the gram staining outcomes. The crystal violet dye, which binds the safranin dye but dissipates when bleached with a bleaching

solution, is responsible for this crimson hue. This is due to the fact that gram-negative bacteria cell walls consist primarily of a lipid layer and have a scant peptidoglycan layer. In addition to this, gram-negative bacteria possess lipoproteins, phospholipids, and lipolysaccharides comprising their outer membrane (Silaban & Simamora, 2018). Bacteria belonging to the genera Pseudomonas and Pseudovibrio are classified as Gram-negative.

Extracellular Enzyme Activity Test

On the basis of the test results, symbiotic microorganisms that produce the enzymes amylase, lipase, and protease have been identified. The amylase-producing bacteria are distinguished by the appearance of a transparent region encircling the paper disc, which has a background coloration of dark blue or purple. Bacteria that produce lipase enzyme are distinguished by the presence of fatty acid deposits encircling the paper disc against a white backdrop. The test outcomes pertaining to microbes that produce protease enzymes are distinguished by the emergence of a transparent region encircling the white-backed paper disc. In contrast, the presence of cellulase enzyme-producing bacteria results in the development of a transparent region encircling the paper disc, which has a yellow underside (Supriyatna et al., 2015).Generally all bacteria can produce enzymes. Because enzymes are one of the components or products of bacterial metabolism (Wantania et al., 2016).

Three isolates of the sponge symbiont microorganisms Xestospongia testudinaria are amylase enzyme producers. Seven isolates of the sponge Acanthella cavernosa are capable of producing the amylase enzyme, bringing the total number of isolates capable of producing the enzyme to ten. As iodine is introduced to the paper disc, the outcomes of the amylase enzyme test are indicated by a transparent region. By introducing iodine solution, the capacity of bacteria to hydrolyze starch is evaluated. The manifestation of this is the disappearance of iodine-colored starch, denoted by the existence of a transparent zone. A number of bacterial genera, including Bacillus, Clotridium, Bacteriodes, Lactobacillus, Micrococcus, Thermus, and Actinomycetes, are well-known for their ability to produce the amylase enzyme (Asadullah, 2014).

Extracellular enzyme assays have identified thirty-one sponge symbiont microorganisms that possess the ability to generate lipase enzymes. As an indication of bacterial capability to produce lipase enzymes, fatty acid deposits accumulate around the paper disk. The percentage of lipase enzymes produced by bacteria was the highest in this study compared to other enzymes; therefore, sponge symbiont bacteria produce a greater quantity of lipase enzymes than other extracellular enzymes, according to the results of extracellular enzyme assays. According to (Asadullah, 2014), lipase enzymes can be produced by various bacterial genera, including Bacillus, Pseudomonas, and Burkholderia.

The subsequent information indicates that two bacterial isolates possess the ability to generate protease enzymes. Protease-producing bacteria are discernible in the transparent zone that encircles the paper disc. The sodium agar medium is supplemented with 1% skim milk. Skim milk proteins are hydrolyzable by bacteria into amino acids, digopeptides, and short chain peptides (Setyati & Subagiyo, 2012). Protease enzyme activity is observed in bacteria belonging to various genera, including Bacillus and

p-ISSN: 2442-9481
e-ISSN: 2685-7332

Pseudomonas. The results of the cellulase enzyme assay indicated that a single bacterial isolate was capable of producing the cellulase enzyme. The outcome of the cellulase enzyme assay is indicated by the development of a transparent region encircling the paper disc. The iodine-treated CMC media produce a distinct zone, which can be observed upon addition. CMC media that have been hydrolyzed and inundated with iodine solution will not stain. In agar media, iodine is utilized as an indicator of enzyme degradation. A number of bacterial genera possess cellulase enzyme activity; for instance, Bacillus and Pseudomonas bacteria are capable of producing cellulase enzymes (Sholihati et al., 2015). Bacteria also use protein for metabolic processes (Suenan et al., 2021).

Isolate code TB2-02-7.1 (Amylase) contained the bacterial isolate with the highest activity, followed by TB2-02-7.3 (Protease), TB1-01-1.2 (Lipase), and TB2-02-4.1 (Cellulase). Multienzyme-producing bacteria also exist; bacteria with the isolate code TB2-02-7.3 are capable of producing lipase, protease, and amylase enzymes. Bacillus is a genus of microorganisms capable of producing extracellular enzymes. Organic substances that Bacillus is capable of degrading include agar, glucose, cellulose, hydrocarbons, and protein. Carotenoids can be produced by symbiont microorganisms in addition to enzymes. Carotenoids, which are classified as tetraterpenoids, are organic metabolism molecules (Ngginak et al., 2020). Enzymes, similar to other chemical compounds, are susceptible to alterations in their chemical structure in response to treatments like heating (Ngginak. et al., 2020).

CONCLUSION

The research findings indicate that a total of 47 bacterial isolates were obtained from the Tablolong beach sponge. Notably, a subset of these isolates exhibited the capability to synthesise extracellular enzymes. A total of ten bacterial isolates exhibited amylase enzyme activity, with isolate TB2-01-7.1 demonstrating the highest activity as indicated by a 36 mm clear zone diameter. Protease enzyme activity was detected in three bakery isolates, with the bacterial isolate TB2-02-7.3 exhibiting the highest activity as measured by a clear zone diameter of 20 mm. A total of 31 bacterial isolates are capable of producing lipase enzyme activity; among these, isolate TB1-01-1.2 exhibits the highest activity, as indicated by a clear zone diameter of 20 mm. In contrast, the cellulase enzyme was present in a solitary bacterial isolate designated TB2-02-4.1, measuring 4 mm in diameter. The TB2-02-7.3 isolate of bacteria exhibits multienzymatic activity, specifically through the production of lipase, protease, and amylase enzymes. On the basis of their capabilities, it is hypothesized that these bacteria are members of the Bacillus genus.

REFERENCES

Arfah, R. A., Patong, A. R., Ahmad, A., & Djide, M. N. (2014). Isolasi dan Identifikasi Bakteri Termofil Penghasil Amilase dari Sumber Air Panas Lejja Sulawesi Selatan. *Al-Kimia*, *2*(2), 36–46. https://doi.org/https://doi.org/10.24252/al-kimia.v2i2.1644

Asadullah, M. (2014). Isolasi Bakteri Amilolitik dari Bekatul dan Uji Kemampuan untuk Produksi Enzim Amilase Kasar pada Berbagai Jenis Media Produksi. *Central Library (Jurusan Kimia Fakultas Sains dan Teknologi UIN)*, *1*(1), 1–98. https://doi.org/http://etheses.uin-malang.ac.id/8263/1/09630049.pdf

Belang, A., Ngginak, J., & Nge, S. (2018). Analisis Protein, Tekstur dan Rasa Ice Cream Berbahan Dasar Rumput Laut Merah (Kappaphycus alvarezii). *Jurnal Ilmiah Teknologi Pertanian*, *3*(1), 269–276.

Cita, Y. P., Radjasa, O. K., & Sudharmono, P. (2015). Aktivitas Antibakteri Isolat bakteri X2 yang Berasosiasi Spons Xestospongia testudinaria dari Pantai Pasir Putih Situbondo terhadap Bakteri Pseudomonas aeruginosa (Antibacterial Activity of Isolates of Bacteria X2 Associated with Sponge Xestospongia testu. *Fakultas Kedokteran Universitas Indonesia. Diterima*, *14*(2), 206–211.

Damar, P. M., Dwiloka, B., Radjasa, O. K., & Ngginak, J. (2021). Opportunity dnd Benefits of Functional Food from the Sea : A REVIEW. *Jurnal Sains Natural*, *11*, 87–95. https://doi.org/https://doi.org/10.31938/jsn.v11i2.297

Haedar., Sadarun, B., & Palupi, R. . (2016). Potensi Keanekaragaman Jenis dan Sebaran Spons di Perairan Pulau Saponda Laut Kabupaten Konawe. *Sapa Laut*, *1*(1), 1–9. https://doi.org/http://dx.doi.org/10.33772/jsl.v1i1.923

Hutasoit, N., Ina, P. T., & Permana, I. D. G. M. (2017). Optimasi pH dan suhu pada aktivitas enzim lipase dari biji kakao (Theobroma cacao L.) berkapang. *Jurnal Ilmu Dan Teknologi Pangan*, *5*(2), 95–102. https://doi.org/https://ojs.unud.ac.id/index.php/itepa/article/view/27504

Marzuki, I., Noor, A., La Nafie, N., & Djide, N. M. (2018). Isolasi dan Identifikasi Bakteri Shimbion Spons Penghasil Enzim Amilase asal Pantai Melawai Balikpapan. *Jurnal Ilmiah "Dr. Aloei Saboe,"* *1*(April), 11–18. https://doi.org/10.17605/OSF.IO/R4JYA

Murtius, W. S., HAri, P. D., & Fiana, R. . (2017). *Isolasi, Seleksi dan Identifikasi Mikroorganisme Potensial Penghasil Enzim Ekstraseluler (Lipase dan Amilase) yang Diisolasi dari Galamai BS (Barang Sisa)* [Universitas Andalas]. http://repo.unand.ac.id/12245/1/Wenny_Surya_Murtius_Universitas_Andalas_PF_Lanjutan-ilovepdf-compressed.pdf

Ngginak., Nge, S. T., Klau, F. F., & Bisilissin, C. L. (2020). Reducing Sugar Content in Siwalan Roomie (Borassus flabellifer L) Before Cooking and Liquid Brown Sugar after Cooking. *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)*, *7*(1), 8–15. https://doi.org/10.31289/biolink.v7i1.2919

Ngginak, J., Rafael, A., Amalo, D., Nge, S. T., & Bisilissin, C. L. S. (2020). Analysis of Content of β-Caroten Compounds in Palm Fruit (Arenga piñata) from Baumata Village. *JAMBURA (Edu Biosfer Journal)*, *2*(1), 1–7. https://doi.org/https://doi.org/10.34312/jebj

Pasodung, A. P., Losung, F., Angkouw, E. D., Lintang, R., Mantiri, D. M. H., & Sumilat, D. A. (2018). Uji Aktivitas Antibakteri Spons Plakortis sp. yang Dikoleksi dari Perairan Bunaken. *Jurnal Pesisir Dan Laut Tropis*, *1*(1), 44–51.

https://doi.org/https://doi.org/10.35800/jplt.6.1.2018.20192

Patty, S. I. (2013). Distribusi Suhu, Salinitas dan Oksigen Terlarut di Perairan Kema, Sulawesi Utara. *Jurnal Ilmiah Platax*, *1*(3), 148–157. https://doi.org/10.35800/jip.1.3.2013.2580

Rambu, E. K., Ngginak, J., & Nitsae, M. (2021). Karakteristik Fisiko Kimia Nori Berbahan Dasar Rumput Laut (Kappaphycus alvarezii) dan Daun Kelor (Moringa oleifer L) Physicochemical. *Jurnal Ilmiah Teknologi Pertanian (AGROTECHNO)*, *6*(1).

Remijawa, S. E., Rupidara, D. N., Ngginak, J., & Radjasa, O. K. (2020). Isolasi dan Seleksi Bakteri Penghasil Enzim Ekstraseluler pada Tanah Mangrove di Pantai Noelbaki. *Enggano*, *5*(2), 164–180. https://doi.org/https://doi.org/10.31186/jenggano.5.2.164-180

Sabdaningsih, A., Budiharjo, A., & Kusdiyantini, E. (2013). Isolasi Dan Karakterisasi Morfologi Koloni Bakteri Asosiasi Alga Merah (Rhodophyta) Dari Perairan Kutuh Bali. *Jurnal Biologi*, *2*(2), 11–17. https://doi.org/https://ejournal3.undip.ac.id/index.php/biologi/issue/view/621

Setyati, A. W. &, & Subagiyo. (2012). Isolasi dan Seleksi Bakteri Penghasil Enzim Ekstraseluler (proteolitik, amilolitik, lipolitik dan selulolitik) yang Berasal dari Sedimen Kawasan Mangrove. *ILMU KELAUTAN: Indonesian Journal of Marine Sciences*, *17*(3), 164–169. https://doi.org/10.14710/ik.ijms.17.3.164-169

Sholihati, A. M., Baharuddin, M., & Santi. (2015). Produksi dan Uji Aktivitas Enzim Selulase dari Bakteri Bacillus subtilis. *Al-Kimia*, *3*(2), 78–90. https://doi.org/https://doi.org/10.24252/al-kimia.v3i2.1672

Siallagan, M. D., Ekowati, C. N., Sumardi., & Rosa, E. (2020). Deteksi Kristal Protein pada Isolat Bacillus sp. dengan Pewarnaan Coomassie Brilliant Blue. *Biospecies*, *13*(2), 46–49. https://doi.org/https://doi.org/10.22437/biospecies.v13i2.9562

Silaban, S. &, & Simamora, P. (2018). Isolasi dan Karakterisasi Bakteri Penghasil Amilase dari Sampel Air Tawar Danau Toba. *EduChemia (Jurnal Kimia Dan Pendidikan)*, *3*(2), 222. https://doi.org/10.30870/educhemia.v3i2.3438

Suenan, L. N., Ledo, M. E. S., & Ngginak, J. (2021). Peningkatan Kualitas Kecap Nira Lontar dengan Penambahan Tepung Tempe Enhancement of Lontar Palm Sauce Quality by Tempeh Flour Addition daerah Nusa Tenggara Timur karena. *Sciscitatio*, *2*(1), 16–21. https://doi.org/https://doi.org/10.21460/sciscitatio.2021.21.43

Supriyatna, H., Jauhari, A. A., & Holydaziah, D. (2015). Aktivitas Enzim Amilase, Lipase, dan Protease dari Larva. *JUrnal ISTEK*, *9*(2), 18–32. https://doi.org/10.3923/pjbs.2004.246.252

Takeltanu, D., Ngginak, J., & Manu, T. S. . (2018). Keanekaragaman dan Kelimpahan Jenis Gastropoda di Pantai Sahan Kabupaten Timor Tengah Selatan. In *Seminar Nasional Biologi dan Pendidikan Biologi UKSW: Vol. ISBN 978-6* (Issue Penelitian, Penerapan dan Pembelajaran Biologi dalam Menghadapi Tantangan Abad 21). https://repository.uksw.edu/handle/123456789/15030

Wantania, L. L., Ginting, E. L., & Wullur, S. (2016). Isolasi Bakteri Simbion Dengan Spons Dari Perairan Tongkeina, Sulawesi Utara. *Jurnal LPPM Bidang Sains Dan Teknologi*, *3*(1), 57–65. https://doi.org/https://doi.org/10.35801/jlppmsains.3.1.2016.15208

Siallagan, M. D., Ekowati, C. N., Sumardi, & Rosa, E. (2020). Deteksi Kristal Protein pada Isolat Bacillus sp. dengan Pewarnaan Coomassie Brilliant Blue. *Biospecies*, *13*(2), 46–49. https://doi.org/https://doi.org/10.22437/biospecies.v13i2.9562

Silaban, S. &, & Simamora, P. (2018). Isolasi dan Karakterisasi Bakteri Penghasil Amilase dari Sampel Air Tawar Danau Toba. *EduChemia (Jurnal Kimia Dan Pendidikan)*, *3*(2), 222. https://doi.org/10.30870/educhemia.v3i2.3438

Suenan, L. N., Ledo, M. E. S., & Ngginak, J. (2021). Peningkatan Kualitas Kecap Nira Lontar dengan Penambahan Tepung Tempe. *Sciscitatio*, *2*(1), 16–21. https://doi.org/https://doi.org/10.21460/sciscitatio.2021.21.43

Supriyatna, H., Jauhari, A. A., & Holydaziah, D. (2015). Aktivitas Enzim Amilase, Lipase, dan Protease dari Larva. *Jurnal ISTEK*, *9*(2), 18–32. https://doi.org/10.3923/pjbs.2004.246.252

Takeltanu, D., Ngginak, J., Manu, T. S. (2018). Keanekaragaman dan Kelimpahan Jenis Gastropoda di Pantai Sahan Kabupaten Timor Tengah Selatan. (Prosiding Seminar Nasional Biologi dan Pendidikan Biologi : Penelitian, Penerapan dan Pembelajaran Biologi dalam Menghadapi Tantangan Abad 21). ISBN 978-602-61913-1-1. 44–51. https://repository.uksw.edu/handle/123456789/15030

Wantania, L. L., Ginting, E. L., & Wullur, S. (2016). Isolasi Bakteri Simbion Dengan Spons Dari Perairan Tongkeina, Sulawesi Utara. *Jurnal LPPM Bidang Sains Dan Teknologi*, *3*(1), 57–65. https://doi.org/https://doi.org/10.35801/jlppmsains.3.1.2016.15208

How To Cite This Article, with *APA style* :
Dima, L.A.M., Rafael, A., Nge, S.T.M., Radjasa, O.K., Manalu, T.S.J., & Ngginak J. (2023). Isolation and Selection of Extracellular Enzymes in Sponge Symbiont Bacteria (Porifera: Demospongiae) from Tablolong Beach. *Jurnal Pembelajaran dan Biologi Nukleus*, 9(3), 727-742. https://doi.org/10.36987/jpbn.v9i3.5222