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Isolation and Characterization of Superior Symbiotic Bacterial Isolates from the Oenggae Sea with Potential as Antibacterial

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Abstract: Sponges are one of the marine biota that have a symbiotic relationship with bacteria which can produce secondary metabolites as antimicrobials. The main aim of this research was to isolate and identify bacteria that had potential as antibacterials against multi-drug resistant test bacteria. The sponsor samples used in this research were *Stylissa massa*, Salvador Dali, and Angelas sp. The methods used were bacterial isolation, morphological and biochemical characterization, molecular identification, antibacterial tests, and molecular identification. The results of the isolation and characterization of superior symbiont bacteria from the sponges *Stylissa massa*, Salvador Dali, and Angelas sp obtained 85 isolates which were purified with different morphological characters. The results of biochemical tests on TSIA and SCA media, of the 85 isolates, 39 were positive on TSIA media and were able to ferment glucose. The test results on SCA media from 85 isolates were 15, positive on SCA media and the isolates were able to use citrate as a carbon source. Antibacterial test results with the test bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. The test results showed that 15 isolates could inhibit the growth of *Escherichia coli* bacteria, 17 isolates could inhibit *Staphylococcus aureus*, 34 isolates could inhibit *Bacillus subtilis* and 12 isolates could inhibit *Pseudomonas aeruginosa*. The results of the 16S rRNA molecular identification of 6 species of isolates showed that isolates S3C1, S3N6, and S3N7 had genetic similarities to *Bacillus velezensis*, S3C2 was similar to *Bacillus vallimortis*, SMZ16 was similar to *Bacillus subtilis* and SMZ1 was similar to *Lysinibacillus macrolides*. Optimization results of bacterial isolates S3N6 and S3N7 in the media glucose and NACL were found to be optimal for both isolates at a concentration of 3%. The novelty of this research was that the researchers obtained 4 isolates that could inhibit the test bacteria which could be developed for testing bioactive compounds.

Keywords: Antibacterial, Media Optimization, Sea Sponge, 16S rRNA

Introduction

Indonesia is one the countries with the largest island with an area of 3.1 million km^2 and has very high biodiversity. One of the high levels of biodiversity is found in marine diversity such as mangroves, coral reefs, and seagrass beds, along with other groups of marine biotas such as fisheries, mollusks, crustaceans, sponges, algae, and turtles (Sains *et al*., 2005). East Nusa Tenggara (NTT) is said to be one of the archipelagic provinces in Indonesia with a sea area of around 200,000 km². This vast expanse of the sea makes the NTT sea abundant in potential marine resources from fisheries, seaweed, coral reefs, and sponges (Taylor, 2007). Rote Ndao Regency is one of the districts located in NTT province which has a sea with a high diversity of sponges. Sponges are one of the main components and have existed for millions of years as a structural and functional part of marine ecosystems (Cárdenas *et al*., 2012). Sponges are a suitable niche for microorganisms such as bacteria to carry out associations in the form of mutualistic symbiosis (Judianti *et al*., 2014). The relationship between sponges and microorganisms is a symbiotic mutualism where the sponge provides a microhabitat for bacteria and the sponge receives protection from the production of bioactive compound products to maintain the life of the sponge from

various types of predators and pathogens originating from other microorganisms (Grossart *et al*., 2013). Research on the exploration and isolation of bacteria in symbiosis with sponges is increasingly being carried out to obtain superior candidates for producing bioactive compounds as antibacterials. Several studies have reportedly isolated symbiotic bacteria which can be useful as antibacterial, antitumor, antimalarial, and antitumor (Kita *et al*., 2013). The search for bioactive compounds is now directed towards communities of microorganisms in sponge tissues as symbionts. Marine microorganisms have contributed most of the bioactive compounds. Microorganisms can produce the same metabolite compounds as their hosts (Proksch *et al*., 2002). Bacteria are able to grow more quickly so that bioactive compounds can be produced more easily, quickly, and in large quantities on a biotechnological scale than culturing the sponge itself (Rua *et al*., 2014). Based on the above background, this research aimed to isolate and molecularly identify bacteria associated with the sponges *Stylissa massa*, Salvador Dali, and Angelas sp from Oenggae Island which had the potential to act as antibacterials against pathogenic bacteria.

Materials and Methods

Sponge Collection and Bacterial Isolation

Sponge specimens were collected from the waters of Oenggae, Rote Island, East Nusa Tenggara, Indonesia from a depth of approximately 3-30 m. Sponge specimens were stored in a cool box with ice until the bacterial isolation process was carried out. Each unidentified sponge was stored in 70% ethanol for identification. The sponge specimens were washed using sterile seawater three times, to separate dirt attached to the surface of the sponge.

Isolation of Bacteria Associated with Sponges

Isolation of symbiotic bacteria was carried out using the pour method. The isolated sponge samples were crushed and then diluted (Wibowo *et al*., 2023). The next stage of bacterial isolation was carried out using the dilution method (10-1 to 10-9). 100 mL of sample suspension from three dilutions (10-7, 10-8, 10-9) was poured and spread on a petri dish containing Zobell Marine Agar media and incubated at 31°C for 1×24 h. Next, morphological observations and purification of the isolate were carried out.

Biochemical Test and Gram Staining

Biochemical characterization was carried out using TSIA and SCA media testing. The Gram test was carried out using 3% KOH and the Gram staining test. Bacterial colonies from the culture media were taken using a round tube and emulsified on a glass slide in a 3% KOH suspension. The gram staining test was carried out using crystal violet staining solution, Lugol's iodine, alcohol, and safranin solution.

Screening for Antagonistic Activity Against Antibiotic-Resistant Pathogenic Bacteria

Sponge symbiotic bacterial isolates were screened to test antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus aureus* (MRSA), *Bacillus subtilis* and *Escherichia coli*. All pathogenic strains were rejuvenated onto Zobell Marine Agar media for 24 h at 32°C before bioassay.

Identification of Potential Bacteria Isolates Based on the 16S rRNA Gene

Bacterial DNA isolation of superior symbiont bacterial isolates was carried out using a molecular identification method using the 16S rRNA coding gene. Next, the 16S rRNA identification data was analyzed by BLAST to identify bacterial species and measure phylogeny (evolutionary relationships). Phylogenetic measurements and genetic relationship analysis were measured using the MEGA X application (Marchesi *et al*., 1998).

Bacterial Growth Curve

Measurement of the growth curve of bacterial isolates S3N6 and S3N7 was measured using the spectrophotometric method. 10% of the inoculum was added to Zobell Marine Broth medium and incubated. The optimum production time for the bacterial growth phase was determined successively by carrying out measurements using a spectrophotometer with a wavelength of 600 nm every 4 h for 72 h. The data obtained were analyzed and made into graphs.

Optimization of Bacterial Growth Media Conditions

Optimizing the growth of bacterial isolates S3N6 and S3N7 was carried out by measuring growth optimization on Zobell marine broth + Glucose media (Maraziha 2020), Zobell marine broth + Sodium Chloride, measurements were carried out at different concentrations of glucose and sodium chloride, namely 1, 2 and 3% and (Zhao *et al*., 2013).

Results

Isolation, Morphological Characterization, and Biochemical Tests

Results of sponge sampling carried out from the sea waters of Rote Oenggae, Rote Ndao district, East Nusa Tenggara Province, Indonesia, using the diving method. The sponges sampled in the research were *Stylissa massa*, Salvador Dali, Angelas sp. The results of sponge characterization indicated that there were 3 types of

sponges. The results of the sponge characterization in Fig. 1. The sponge samples were then prepared for isolation of bacteria associated with the samples. The results of the isolation and characterization of superior symbiont bacterial isolates from 3 types of marine sponges resulted in 86 isolates with different characteristics (Table 1) and screening tests of 86 superior symbiont bacterial isolates against the test bacteria *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* (Table 2).

The bacterial colonies that grew were then screened and morphologically observed, followed by biochemical identification including TSIA, SCA, and Gram staining tests. For morphological observation data, biochemical tests and gram staining in Table 1.

Fig. 1: Results of sponge sample collection: A. *Stylissa massa*, B. Salvador Dali, C. Angelas sp

Table 1: Morphological characterization and biochemical tests

No.	Isolate Code	Colour	Form	Margin	Elevation	Gram	TSIA	SCA
1	SMZ 1	White	Circular	Entire	Convex	+	+	-
2	SMZ 2	White	Circular	Entire	Convex	-	+	-
3	SMZ 3	White	Circular	Entire	Convex	+	+	-
4	SMZ 4	White	Irregular	Undulate	Convex	+	+	+
5	SMZ 5	White	Irregular	Entire	Convex	-	-	-
6	SMZ 6	White	Irregular	Undulate	Convex	-	-	-
7	SMZ 7	White	Circular	Entire	Convex	-	-	-
8	SMZ 8	White	Circular	Entire	Convex	-	-	-
9	SMZ 9	White	Circular	Entire	Convex	+	+	-
10	SMZ 10	White	Irregular	Undulate	Convex	-	+	-
11	SMZ 11	White	Circular	Entire	Convex	+	+	-
12	SMZ 12	White	Rhizoid	Filamentous	Convex	+	-	+
13	SMZ 13	White	Rhizoid	Filamentous	Convex	-	-	+
14	SMZ 14	White	Irregular	Undulate	Convex	+	-	+
15	SMZ 15	White	Rhizoid	Rhizoid	Convex	-	-	+
16	SMZ 16	White	Irregular	Undulate	Convex	+	+	-
17	SMZ 17	White	Irregular	Undulate	Convex	-	-	-
18	SMZ 18	White	Circular	Entire	Convex	+	+	-
19	SMZ 19	White	Irregular	Undulate	Convex	+	+	-
20	SMZ 20	White	Circular	Entire	Convex	+	-	-
21	SMZ 21	White	Circular	Entire	Convex	+	+	-
22	SMZ 22	White	Circular	Entire	Convex	+	+	-
23	SMZ 23	White	Circular	Entire	Convex	+	-	-
24	SMZ 24	White	Irregular	Undulate	Convex	+	-	-
25	SMZ 25	White	Rhizoid	Rhizoid	Convex	+	-	-
26	SMZ 26	White	Irregular	Undulate	Convex	+	-	-
27	SMZ 27	White	Irregular	Undulate	Convex	+	-	-
28	SMZ 28	White	Circular	Entire	Convex	+	-	-
29	SMZ 29	White	Irregular	Undulate	Convex	+	-	-
30	SMZ 30	White	Circular	Entire	Convex	+	-	-
31	SMZ 31	White	Circular	Undulate	Convex	+	-	-
32	SMZ 32	White	Circular	Entire	Convex	+	-	-
33	SMZ 33	White	Irregular	Undulate	Convex	+	-	-
34	SMZ 34	White	Circular	Undulate	Convex	+	-	-
35	SMZ 35	White	Circular	Undulate	Convex	+	-	-
36	SMS 1	White	Irregular	Undulate	Convex	+	-	-
37	SMS 2	White	Circular	Undulate	Convex	+	-	-
38	SMS 3	White	Irregular	Undulate	Convex	+	-	-
39	SMN 1	White	Circular	Entire	Convex	+	-	-

Table 1: Continue

40	SMN 2	White	Rhizoid	Filamentous	Flat	+	-	-
41	SMC 1	White	Rhizoid	Rhizoid	Convex	+	-	-
42	SMC 2	White	Rhizoid	Rhizoid	Convex	+	-	+
43	SMC 3	White	Irregular	Lobate	Convex	+	+	-
44	SMC 4	White	Circular	Entire	Convex	+	-	-
45	SMC 5	White	Irregular	Rhizoid	Convex	+	-	+
46	SMC 6	White	Circular	Entire	Convex	+	-	+
47	SMC 7	White	Circular	Entire	Convex	+	-	+
48	SMC 8	White	Circular	Undulate	Convex	+	-	+
49	SMC 9	White	Circular	Undulate	Convex	+	-	+
50	S2Z 1	White	Circular	Entire	Convex	+	-	-
51	S2Z 2	White	Circular	Entire	Convex	+	-	-
52	S2Z 3	White	Circular	Entire	Convex	+	-	-
53	S2Z 4	White	Circular	Undulate	Convex	+	-	-
54	S2Z 5	White	Circular	Undulate	Convex	+	-	-
55	S2Z 6	White	Irregular	Undulate	Convex	+	-	-
56	S2Z 7	White	Circular	Undulate	Convex	+	+	-
57	S2Z 8	White	Rhizoid	Filamentous	Convex	+	-	-
58	S2Z 9	White	Irregular	Lobate	Convex	+	-	-
59	S2Z 10	White	Irregular	Rhizoid	Convex	+	-	-
60	S2Z 11	White	Irregular	Undulate	Convex	+	-	-
61	S2Z 12	White	Circular	Undulate	Convex	-	-	-
62	S2Z 13	White	Irregular	Undulate	Convex	+	-	-
63	S2Z 14	White	Circular	Entire	Convex	+	-	-
64	S2Z 15	White	Circular	Undulate	Convex	+	-	-
65	S2Z 16	White	Circular	Entire	Convex	+	-	-
66	S2Z 17	White	Circular	Entire	Convex	+	-	-
67	S2S 1	White	Irregular	Undulate	Convex	+	-	-
68	S2S 2	White	Circular	Undulate	Convex	+	-	-
69	S2N	White	Circular	Entire	Convex	+	+	-
70	S3S 1	White	Circular	Entire	Convex	+	-	-
71	S3S 2	White	Circular	Entire	Convex	+	-	+
72	S3S 3	White	Circular	Entire	Convex	+	-	+
73	S3S 4	White	Circular	Entire	Convex	+	-	+
74	S3S 5	White	Circular	Entire	Convex	+	-	-
75	S3S 6	White	Irregular	Undulate	Convex	+	-	-
76	S3S 7	White	Circular	Entire	Convex	+	-	-
77	S3C 1	White	Circular	Entire	Convex	+	-	-
78	S3C 2	White	Circular	Entire	Convex	+	+	-
79	S3C 3	White	Circular	Entire	Convex	+	+	+
80	S3N 1	White	Circular	Entire	Convex	+	-	+
81	S3N 2	White	Circular	Entire	Convex	+	+	-
82	S3N 3	White	Circular	Entire	Convex	+	-	-
83	S3N 4	White	Circular	Entire	Convex	+	-	-
84	S3N 5	White	Circular	Entire	Convex	+	+	-
85	S3N 6	White	Circular	Undulate	Convex	+	+	-
86	S3N 7	White	Rhizoid	Filamentous	Convex	+	-	+

Antagonist Test of the Test Bacterial Isolate Against the Test Bacteria

Bacterial isolate testing was carried out using the disc diffusion method to determine the potential of each isolate to inhibit the growth of the test bacteria. The antibacterial potential of each isolate was observed and the inhibition zone formed was measured. The results of testing bacterial isolates against the test bacteria in Table 2.

Potential bacterial isolates were identified by molecular16S rRNA to determine the bacterial species. The results of the identification of 6 potential bacterial isolates in inhibiting the test bacteria in Table 2. The results of the dendrogram analysis and measurement of the genetic distance between the identified species (Table 3, Fig. 2).

Table 2: Potential of superior bacterial isolates against test bacteria

		Obstacles zone (cm)			
No.	Isolate code	*Escherichia coli*	*Staphylococcus aureus*	*Bacillus subtilis*	*Pseudomonas aeruginosa*
1	SMZ 1	-	6,5	2	0,1
2	SMZ 2	-	1	-	-
3	SMZ 3	-	-	-	0,1
4	SMZ 4	-	-	1,5	-
5	SMZ 9	-	-	-	0,1
6	SMZ 11	-	-	-	0,1
7	SMZ 12	-	2,2	-	-
8	SMZ 14	-	-	-	0,1
9	SMZ 16	-	1	2,5	-
10	SMZ 17	-	-	5,5	-
11	SMZ 18	-	-	5,4	-
12	SMZ 20	-	-	1,2	-
13	SMZ 22	-	-	0,7	-
14	SMZ 23	1	-	-	-
15	SMZ 26	-	-	2,5	-
16	SMZ 27	1,3	-	-	-
17	SMZ 32	1,4	3,1	4,5	-
18	SMZ 33	-	-	-	-
19	SMZ 34	-	2,9	-	-
20	SMS 1	-	-	2	-
21	SMS 2	-	-	4,5	-
22	SMS 3	-	-	2,5	-
23	SMN 1	-	-	1,7	-
24	SMC 1	-	-	6,5	-
25	SMC 2	1	1,5	0,1	-
26	SMC 3	1,2	-	7	-
27	SMC 4	-	-	4	-
28	SMC 5	-	-	5	-
28	SMC 6	-	-	4,2	-
29	SMC 7	1	-	3,9	-
30	SMC 8	1,3	-	1	-
31	SMC 9	-	-	0,1	-
32	S2Z 1	-	0,1	0,8	-
33	S2Z 2	-	-	1,5	-
34	S2Z 3	-	-	6	-
35	S2Z 8	-	-	2,7	-
36	S2Z 13	-	-	1,5	-
37	S2Z 16	1,3	-	-	-
38	S2S 2	1,4	-	-	-
39	S2N	-	-	2,2	-
40	S3S6	-	-	3	-
41	S3C1	4,4	5,5	3,3	4,4
42	S3C2	2,5	6	3,8	3
43	S3C3	-	1,7	-	-
44	S3N1	0,1	0,4	-	-
45	S3N2	-	2,3	-	4,4
46	S3N3	-	5	-	-
47	S3N4	-	-	7	-
48	S3N5	1,5	2,1	-	0,1
49	S3N6	3,5	4,4	8,2	5,3
50	S3N7	3	4,6	2,3	2,8

Table 3: Results of 16 S rRNA gene analysis

Code isolate	Scientific name	Max score	Total	E-Value	Length	Similarity	Number accession
S3C1	*Bacillus valezensis* strain NRRL B-41580	2689	1484	0	1512	99.46	KY694464.1
S3N7	*Bacillus valezensis* strain NRRL B-41580	2726	1483	0	1508	99,86	KY694464.1
S3N6	*Bacillus valezensis* strain NRRL B-41580	2704	1464	0	1508	100	KY694464.1
S3C2	*Bacillus vallimortis* strain DSM 11031	1488	1477	0	1494	99.72	NR_024696.1
SMZ16	*Bacillus subtilis* strain NCIB 3610	2732	1497	0	1988	99.66	CP020102.1
SMZ 1	*Lysinibacillus macrolides* strain DSM 54	2723	1485	0	1517	99.79	KY643638.1

Table 4: Results of genetic distance measurements

	1	2	3	4	5
Bacillus_valezensis_strain_NRRL_B-41580					
Bacillus_valezensis_strain_NRRL_B-41580_2	3.0683				
Bacillus_vallimortis_strain_DSM_11031	5.1524	6.1212			
Bacillus_subtilis_strain_NCIB_3610	5.1173	5.7585	3.4726		
Lysinibacillus_macrolides_strain_DSM_54	8.7118	5.1664	6.6673	8.9228	

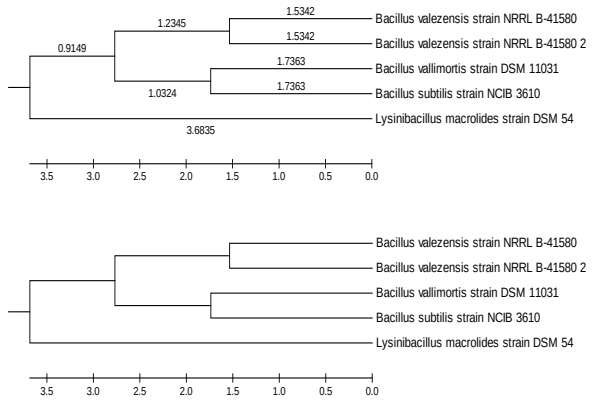

Fig. 2: Results of Dendrogram analysis and measurement

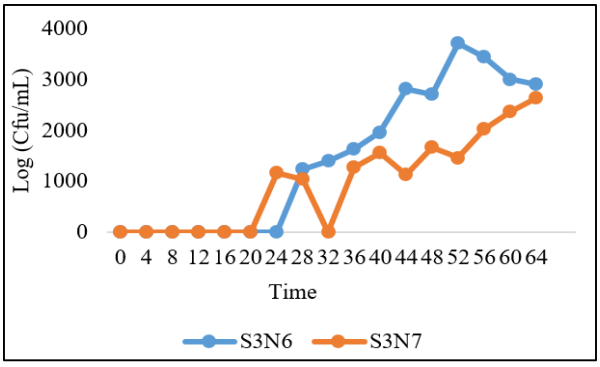

Fig. 3: Results of growth curve measurements for Isolates S3N6 and S3N7

Growth Curve and Media Optimization

Two superior symbiont bacterial isolates, namely S3N6 and S3N7, which had antibacterial potential and inhibited all test bacteria, were continued for growth curve measurements. The results of measuring the growth curves of isolates S3N6 and S3N7 in Fig. 3. Next, these isolates were grown on ZMB + NaCl and ZMB + Glucose media to see the optimization of bacterial growth (Figs. 4-5).

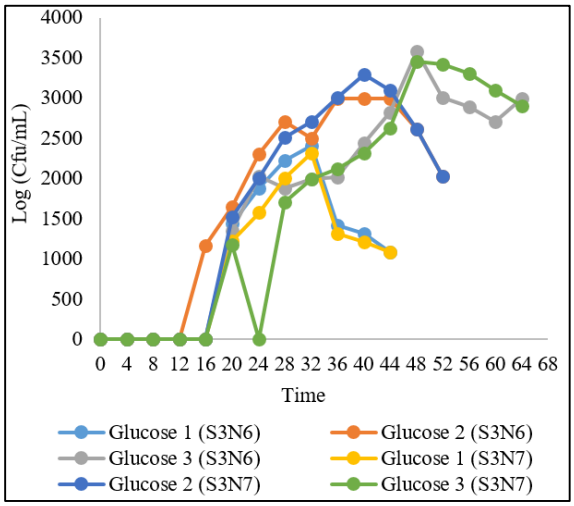

Fig. 4: Optimization of media Zobell marine broth + glucose; Ket: Glucose 1= 1%, glucose 2 = 2%, glucose 3 = 3%

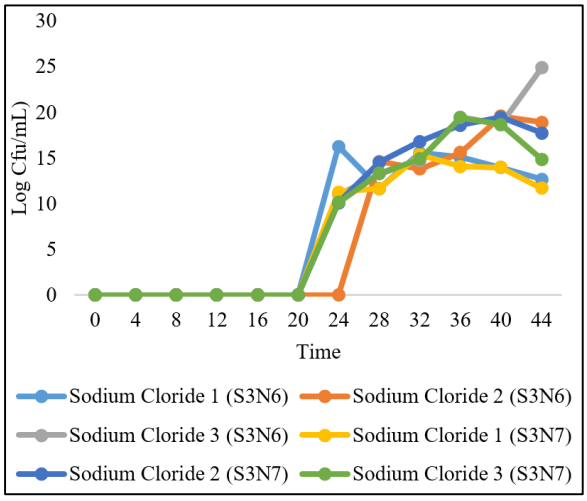

Fig. 5: Optimization of Growth on Zobell marine Broth + Sodium Chloride media; Note: Sodium Clorida 1 = 1%, Sodium Chloride 2 = 2%, Sodium Chloride 3 = 3%

Discussion

The results of the colony morphology characterization of 86 isolates of superior symbiont bacteria were found to have different characteristics. The total colony observation was that eighty-six isolates were white. The characterization of the colony shape of superior bacterial isolates was predominantly circular, with 53 isolates, 24 irregular isolates, and 9 rhizoid isolates, while for margin observations there were 41 entire isolates, 32 undulate isolates, 7 rhizoid isolates, and 6 filamentous isolates. The results of colony observations based on the elevation of superior symbiont bacterial isolates were found to have convex and flat elevations. The results of gram testing on eighty-six bacterial isolates isolated from sponges associated with sponges showed that 75 isolates were gram-positive bacteria and 11 isolates were gram-negative bacteria. From the shape observations, it was found that 39 isolates were coccus-shaped and 47 isolates were bacillus-shaped. From the results of biochemical tests including the Triple Sugar Iron Agar (TSIA) test, 39 isolates were able to ferment glucose and 47 isolates were unable to ferment glucose. Simmon Citrate Agar (SCA) test results showed that 15 isolates were able to use citrate as a carbon source and 71 isolates were unable to use citrate as a carbon source. Research Pardosi (2022) on the characterization and identification of SM4 bacterial isolates from the mass stylissa sponge as producers of antimicrobial compounds against pathogenic bacteria found that these isolates were positive in the TSIA and SCA tests. The results of testing superior symbiont isolates showed that 50 bacterial isolates had the potential to inhibit the test bacteria. From the test results, it was found that 15 isolates could inhibit the growth of *Escherichia coli* bacteria, 17 isolates could inhibit *Staphylococcus aureus*, 34 isolates could inhibit *Bacillus subtilis* and 12 isolates could inhibit *Pseudomonas aeruginosa*. One form of response shown by bacteria was the formation of metabolite compounds as a form of defense against other bacteria and to avoid toxic compounds that had the potential to be dangerous to these bacteria (Presson *et al*., 2022).

Molecular identification of symbiotic bacterial isolates was known from 5 isolates identified as being in the genus Bacillus and 1 isolate belonging to the genus *Lysinibacillus*. The results of BLAST analysis of bacterial isolates S3C1, S3N6, and S3N7 are similar to the species *Bacillus velezensis*, S3C2 was similar to *Bacillus vallimortis*, SMZ16 is similar to *Bacillus subtilis* and SMZ1 is similar *to Lysinibacillus macrolides*. This was in accordance with the color characterization, it was known that the five isolates are gram-positive bacteria in the form of bacilli and have similar characteristics. Research (Wibowo *et al*., 2023) found four bacteria of the genus *Bacillus* isolated from marine sponges. Dendrogram analysis of bacterial isolates that had antibacterial potential was analyzed using MEGA. This result showed the similarity of nucleotides. Group 2, namely isolate SMZ 1, was known to belong to the genus *Lynibacillus*. The results of genetic distance measurements were carried out using the MEGA *Bacillus valezensis* with the same strain. The results of the farthest genetic distance analysis showed that isolates S3C1 and SMZ1 had the farthest genetic distance with a scale of 8.9228, this indicated that these isolates belong to different genera, namely the *Bacillus* and *Lynibacillus* genera. The results of the furthest genetic distance analysis indicate that the genomes and nucleotide sequences of these bacteria were similar even though they were isolated from the same waters, namely Oenggae waters. This was in accordance with research (Wibowo *et al*., 2023) which isolated bacteria from sponges and found bacterial isolates of the Bacillus group and had close relatives. Rini *et al*. (2017) found the genus Pseudomonas which had a close phylogenetic tree. Study. Kyule (2022) also reported that isolates isolated from fish and products were found to be closely related species of the genus *Bacillus*.

Based on the graph measuring the growth curve of isolates S3N6 and S3N7, it is known that the lag phase occurred at 0-8 h, the exponential phase occurred at 24 h and the stationary phase occurred at 32 h and after that, the cells decreased to enter the death phase. Factors that influenced microbial growth were the availability of nutrients, air, temperature, pH, oxygen, oxidation-reduction potential, the presence of inhibitory substances, and the presence of other microorganisms. The results of optimizing bacterial isolates S3N6 and S3N7 on glucose media showed that both isolates had the highest growth optimization at a media concentration of 3% and the lowest growth optimization at a concentration of 1%. This affected the higher the concentration of the isolated glucose growth phase, the faster and longer it was because glucose was a carbon source that could be used for growth. Meanwhile, measurements of the optimal growth of isolates S3N6 and S3N7 on Zobell Marin Bond + Sodium Chloride media showed that both isolates could grow optimally at concentrations of 3% and as low as 1%. This was influenced by halophilic bacteria, which are a group of microorganisms that can grow in environments with low to high salt levels (Sabdaningsih and Lunggani, 2020). Halophilic bacteria were divided into 3 groups based on their ability to live, namely low halophiles (slight halophiles) that grow optimally in the range of 2-5% NaCl levels, moderate halophiles (moderate halophiles) in the range of 5-20% NaCl levels and extreme halophiles (extreme halophiles) in the range of 20-30%. NaCl content. These bacteria were often found in salt lakes, especially salt pond crystallization tables (Harihastuti and Eddy, 2014). According to Malik *et al*. (2019), the presence of extreme halophilic bacteria on the crystallization table could speed

up the evaporation process which could help optimize the absorption of sunlight. These bacteria also had the ability to accelerate the salt crystallization process and influence the physical condition of the salt.

Conclusion

From the research results, it was concluded that the results of bacterial isolation from the *Stylissa massa*, Salvador Dali, and Angelas Sp sponges showed that 86 isolates were obtained and 50 isolates had the potential to inhibit the test bacteria. The results of the identification of the 16 S rRNA gene showed that the identified isolate belonged to the genus Bacillus and Lysinibacillus. Optimizing the growth of bacterial isolates S3N6 and S3N7 was known to be optimal when adding 3 percent glucose and NaCl.

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Author's Contributions

Lukas Pardosi: The original concept, designed of the research technique, field sampling, data analysis and manuscript approval were done.

Adelya Irawan Manalu and Charles Venirius Lisnahan: Field sampling, engagement of resources and equipment, literature research.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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