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Metabolites of Mangrove Sediment Bacteria from Semarang and Karimunjawa as Anti-Fungal and Antibacterial

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Abstract

Pathogenic bacteria are microorganisms that cause disease in humans because bacteria grow cosmopolitan with human life, both on land and underwater. This research aimed to obtain mangrove sediment bacteria that have the potential as antibacterial and antifungal bacteria from mangrove sediments in Semarang and Karimunjawa waters. Mangrove sediment bacteria that have the potential as antibacterial and antifungal bacteria from Semarang and Karimunjawa waters, as well as identification of their compounds using the GCMS method. Sediment samples were collected from Semarang and Karimunjawa, and bacterial screening and isolation were then carried out. Bacterial isolates were tested with pathogenic fungi Malassezia furfur and Candida albicans. As well as the pathogenic bacteria *Staphylococcus aureus*, *Escherichia coli*, and *Staphylococcus typhi*. Then, the potential bacteria were identified by the molecular DNA method and analyzed by the GC-MS method to determine the secondary metabolite compounds that act as antifungal and antibacterial. The results showed that the bacterial isolation of mangrove sediments from Karimunjawa and Semarang that had antibacterial activity against pathogenic bacteria *E. coli*, *S. aureus*, and *S. typhi* were 3 isolates, namely SKK 1 MA, SKS 2 ISP, and SKS 4 ISP. The 3 isolates also had antifungal activity against the fungal pathogens *Malassezia furfur* and *Candida albicans*. The molecular identification showed that the 3 potential isolates, namely SKK 1 MA, SKS 2 ISP, and SKS 4 ISP, were *Bacillus* sp., *Bacillus cereus*, and *Alcaligenes faecalis*, respectively. Results of the analysis of the content of bacteria as antifungal and antibacterial showed that the bacteria were dominated by the content of unsaturated fatty acids (omega 9) and saturated fatty acids.

Keywords: Anti-bacterial, Antifungal, Mangrove, Molecular, Pathogen, Unsaturated fatty acids, Saturated fatty acids

Introduction

Mangroves are unique and have various benefits, such as being a source of food for marine biota [1], a habitat for marine biota [2], antimicrobial potency [3], tanin [4] and chemical profile [5]. Recently, pathogenic fungal infections are one of the most difficult diseases to treat. The influence of pathogenic components in the emergence of disease is highly dependent on the presence of pathogens, the number of pathogen populations, and the ability of pathogens to cause disease [6], which is the ability to infect (virulence) and the ability to attack the host (aggressiveness) [7], the pathogens adaptation ability, the spread, and the survival [8]. Pathogenic microbes have evolved, one of which is in the development of resistance mechanisms to drug compounds. It is predicted that pathogenic microbial resistance will kill 10 million people by 2050 if there is no immediate action taken. Nature is the main provider of bioactive ingredients needed by humans to cure various diseases [9].

During the last 150 years, more than 100.000 types of natural bioactive compounds have been identified, such as polyketides, alkaloids, non-ribosomal peptides, isoprenoids or phenylpropanoids [10]. Several types of identified natural bioactive compounds are from microorganisms belonging to the mangrove sediment bacteria group, such as *Pseudomona aeruginosa* and *Zhouia amylolytica* strain HN-181, which serve as an antifungal [11]. On the other hand, mangrove sediment bacteria also have the potential as antibacterial agents. On the other hand, actinomycetes bacteria in mangrove sediments in Tapak Village, Tugurejo, Semarang City and Mosquito Island, Karimunjawa, Indonesia, have been found bacteria that have the potential to an antibacterial against pathogenic bacteria of the type *Staphylococcus aureus, Escherichia coli*, and *Listeria monocytogenes* [12]). Natural substances produced by microorganisms are very diverse in structure and are an important source in the search for new drugs for various diseases in

humans, including infections by pathogenic bacteria [13].

Hence, this research aims to determine the potential of sedimentary bacteria from Semarang and Karimunjawa as antibacterial and antifungal, to determine the compounds contained in bacteria that have potential as antibacterial and antifungal using the GCMS method, as well as to identify potential bacterial DNA molecules.

Materials and methods

Mangrove sediment sampling

Sediment samples were taken from 2 different locations, which were mangrove area in Tapak Village, Semarang, as a location with polluted environmental conditions, and a mangrove area on Menjangan Besar Island, Karimunjawa, as a location with unpolluted environmental conditions. The samples were stored in a zip lock and then taken to the Tropical and Marine Science Laboratory, Faculty of Fisheries and Marine Science, Diponegoro University for further investigation.

Isolation of bacteria from mangrove sediment samples

The sediment sample treatment method for bacterial isolation was carried out according to the Davies-Bolorunduro method [14]. Each dilution result was taken 50 µL and flattened on the surface of 5 different medium types, which were Marine Agar (Zobell), International Streptomyces Project 1 (ISP 1) [15], and Humic Acid Vitamin Agar (HVA) [16], and agar medium. Each medium was incubated at a temperature of 29 - 34 ℃ for 1 - 7 days.

The purification of bacterial isolates. The samples that had been spread on MA, HVA, and ISP medium, were then identified to examine the morphology of each isolate that had grown. The morphology identified shape, color, elevation, margin, and size [17]. Antifungal bacteria screening was carried out using the agar plug method with some modifications [18].

Antifungal test

The antifungal test was carried out qualitatively and quantitatively. The qualitative test method uses an agar plug, while the quantitative test uses the disc diffusion method. The pathogenic fungi used in this study were *Malasezia furfur* and *Candida albicans.* Observations of the clear zone were carried out within 24, 48 and 72 h [19].

Qualitative and quantitative antifungal test

Qualitative antifungal test was carried out using the agar plug method based on Messaoudi and Wink [18] with a modification, namely a Petri dish with holes. Meanwhile, the quantitative test was carried out using the paper dish method based on Messaoudi and Wink [18]. The media used in the test is Mueller Hinton Agar (MHA). Subsequent observations on qualitative and quantitative tests were clear zones that formed within 24, 48 and 72 h.

Antibacterial test

Antibacterial tests were carried out qualitatively and quantitatively. The qualitative antibacterial test used the agar plug, while the paper disk or disk diffusion method was used for qualitative [18] and quantitative [20] antibacterial tests. The media used in the test was Mueller Hinton Agar (MHA) and the target pathogenic bacteria including *S. aureus*, *E. coli*, and *S. typhii*. Pathogenic bacteria collected from Microbiology Laboratory, Central Laboratory, Faculty of Medicine, Diponegoro University, Diponegoro National Hospital The clear zone formed was observed in 24 and 48 h.

Target bacterial DNA extraction and 16S rRNA amplification

Bacterial DNA extraction was carried out using the Chelex method, because it is simple and effective with results that are in accordance with the objectives. Amplification of the 16S rRNA gene was carried out by mixing 1 µL of DNA template, primer pair of 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1429R (5'-GGTTACCTTGTTACGACTT-3') [21]. The primers used in DNA amplification using PCR 16S rDNA are Forward (5'-AGAGTTTTGATCMTGGTCAG-3') positions 8-27 and 1492 Reverse (5'-TACGGYTACCTTGTTACGACTT-3'). While the temperature treatment used is as follows: denaturation at 94 °C for 2 min, then 30 cycles (annealing at 50 °C for 40 s, extension at 72 °C for 1 min and denaturation again at 94 °C for 40 s, and 42 °C for 1 min, 72 °C for 5 min and finally 4 °C. PCR products were then electrophoresed using 0.8 % agarose gel. After electrophoresis was completed, visualization was carried

out to observe the DNA bands. The DNA bands formed were then cut and stored in a microcentrifuge tube, then stored at −20 °C.

Sequencing and data processing

The amplified sample was sent to PT. Genetics Science Indonesia for the sequencing process that aimed to determine the sequence of nucleotide bases using the Sanger Deoxy Method. The data from the sequences were edited using MEGA 7.0 software, then the data from the 16s rDNA primer was matched with the data from the NCBI GenBank (ID SUB12695368).

Secondary metabolite-producing gene mapping simulation

To carry out GC MS analysis, the sample is extracted first, namely by: Take a bacterial colony isolate and dissolve it in 1 mL of sterile water in a microcentrifuge tube with a volume of 1.5 mL. Centrifuge for 1 min at 10,000 - 12,000 rpm, then discard the supernatant obtained. Add 200 µL of instance matrix pellet (bacterial precipitate), then vortex and incubate at 56 C using a heat block for 30 min, vortex again at high speed for 10 s, place the tube at 100 °C on the heat block for 8 min, then vortex at high speed for 10 s, centrifuged at 12,000 rpm for 3 min. Gas Chromatography-Mass Spectrometer Analysis or GC-MS analysis was done using GC-MS QP2010S. The number of compounds contained in the extract was indicated by a peak on the chromatogram while the names/types of compounds present were interpreted based on the spectral data of each peak by using the library research method on the GC-MS database [22].

Results and discussion

Antifungal qualitative test

The morphology of isolates from Karimunjawa and Semarang mangrove sediment samples appeared to be circular, irregular, and filamentous from the 17 isolated isolates (**Figure 1**). The antifungal test was carried out qualitatively and quantitatively. The results showed that of the 17 isolates that had been qualitatively tested, there were 8 isolates capable of producing an inhibitory zone against *C. albicans*. Of the 8 isolates, the inhibition zone formed on average appeared at 48 h of observation. Meanwhile, 12 isolates had antifungal activity against *M. furfur*, 4 isolates showed inhibition zones at 24 h of observation, while the others showed inhibition zones at 48 and 72 h of observation. There were 4 isolates that had good antifungal activity against *C. albicans* and *M. furfur,* including SKS 2 ISP, SKS 1 ISP, SKK 2 MA, and SKS 4 HVA isolates. The formation of inhibition zones for both *C. albicans* and *M. furfur* indicated that the 4 isolates had stronger potential of antifungal compounds than the other isolates as shown in **Table 1**.

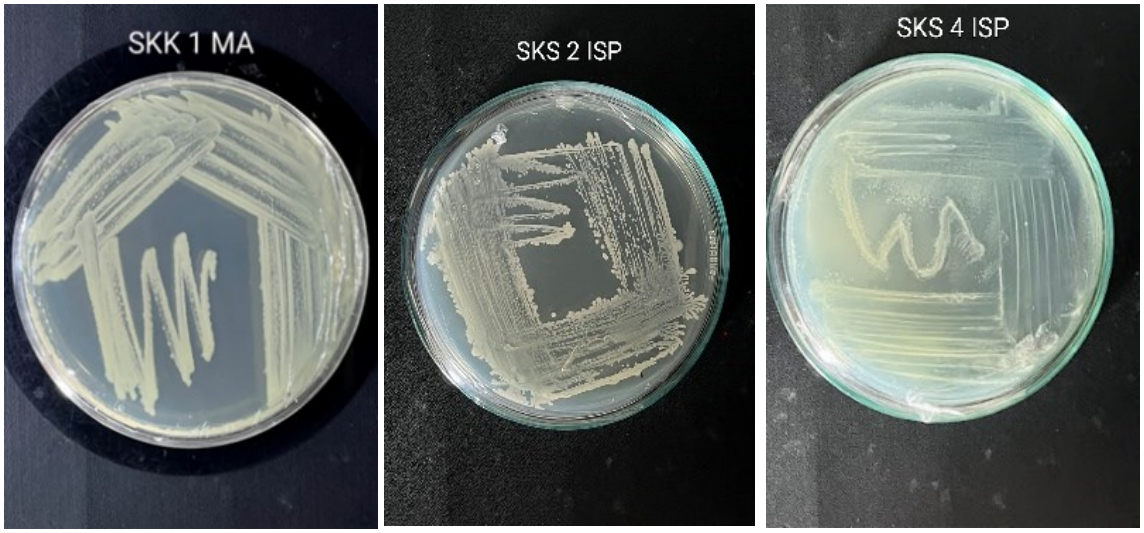

Figure 1 Three isolates from mangrove sediments, namely: SKK 1 MA, SKS2 ISP, SKS4 ISP.

Table 1 Results of antifungal qualitative test.

No	Bacterial code	*Candida albicans*			*Malassezia furfur*		
		24 h	48 h	72 h	24 h	48 h	72 h
1	SKK 1 ISP	-	-	+	-	-	-
2	SKS 2 ISP	-	-	+	-	-	+
3	SKS 1 ISP	-	+	+	-	+	+
4	SKS 4 ISP	-	-	-	-	+	+
5	SKS 3 MA	-	-	-	-	+	-
6	SKK 2 MA	-	-	+	+	+	+
7	SKK 5 MA	-	-	-	+	+	+
8	SKK 6 MA	-	-	-	+	+	+
9	SKS 1 MA	-	-	-	+	+	+
10	SKS 2 MA	-	+	+	-	-	-
11	SKK 4 ISP	-	+	+	-	-	-
12	SKS 1 MA	-	+	+	-	-	-
13	SKK 2 MA	-	-	-	-	-	+
14	SKS 2 HVA	-	-	-	-	-	+
15	SKS 3 HVA	-	-	-	-	+	+
16	SKS 4 HVA	+	+	+	-	-	+
17	SKK 1 HVA	-	-	-	-	-	-

Note: K (Karimunjawa), S (Semarang), ISP (International Streptomyces Project) , MA (Malt Agar), HVA (Humic Vitamin Agar)

Antifungal quantitative test

The antifungal test results of sediment isolates against *C. albicans* and *M. furfur* quantitatively showed that 3 isolates had the highest antifungal activity, namely SKK 1 MA, SKS 2 ISP, and SKS 4 ISP isolates. At 24 h observation, SKK 1 MA, SKS 2 ISP, and SKS 4 ISP isolates had inhibition zones of 5±1.41 mm, 5±1.41 and 3±4.24 mm against *C. albicans* and 4±1.41, 7.5±6.36, 4.5±6.36 mm against *M. furfur*. At 48 h of observation, the 3 isolates showed an increase in the inhibition zone with SKK 1 MA (5.5±2.12 mm), SKS 2 ISP (10±2.82 mm), and SKS 4 ISP (5.5±7.77 mm) against *C. albicans* and SKK. 1 MA (6.5±0.7 mm), SKS 2 ISP (17±0 mm), SKS 4 ISP (12±0 mm) against *M. furfur*. The 3 isolates had antifungal activity because according to Khasanah and Nastiti [23], the presence of a clear zone around the paper disk and an increase in the area of the inhibition zone indicated the presence of antibacterial activity in the bactericidal category. the level of bacterial inhibitory power was categorized into very strong (> 20 mm), strong (10 - 20 mm), moderate (5 - 10 mm), and weak (< 5 mm) [24]. The isolates SKK 1 MA, SKS 2 ISP, and SKS 4 ISP had an inhibition zone of about 5 - 10 mm which indicated that the 3 isolates had moderate antifungal activity as shown in **Table 2**.

Table 2 Results of antifungal test of mangrove sediment isolates against *C. albicans* and *M. furfur* fungi.

No	Bacterial code	Inhibiton zone			
		C. albicans		*M. furfur*	
1	SKK 1 ISP	0±0	0±0	0±0	0±0
2	SKK 3 ISP	0±0	0±0	1.5±2.12	6±0
3	SKK 4 ISP	2.5±3.53	2.5±3.53	0±0	0±0
4	SKS 1 ISP	3±4.24	3.5±4.9	0±0	0±0
5	SKS 2 ISP	5±1.41	10±2.82	7.5±6.36	17±0
6	SKS 4 ISP	3±4.24	5.5±7.77	4.5±6.36	12±0
7	SKK 1 ISP	2±2.82	2.5±3.53	2.5±3.53	3±2.82
8	SKK 1 MA	5±1.41	5.5±2.12	4±1.41	6.5±0.7
9	SKK 5 MA	5±1.41	5.5±2.12	6±1.41	7±0
10	SKK 6 MA	4.5±0.7	5±0	3±0	4.5±0.7
11	SKS 3 ISP	3.5±4.9	3.5±4.94	4±5.65	6±7.07
12	SKS 3 HVA	5.5±0.7	6±1.41	7±0	7±1.41

Note: K (Karimunjawa), S (Semarang), ISP (International Streptomyces Project) , MA (Malt Agar), HVA (Humic Vitamin Agar)

Antibacterial qualitative test

Results of the qualitative antibacterial test showed that the isolated mangrove sediment isolates had an antibacterial activity indicated by the appearance of an inhibition zone. Of the 17 tested isolates, 3 isolates have the most potential as both antibacterial and antifungal, namely isolates SKK 1 MA, SKS 2 ISP, and SKS 4 ISP. The 3 isolates showed antifungal and antibacterial activity. SKK 1 MA has antibacterial activity against *E. coli* pathogens, SKS 2 ISP has antibacterial activity against *B. subtilis*, *E. coli*, and *S. aureus*, and SKS 4 ISP has antibacterial activity against all tested pathogens.

Antibacterial quantitative test

The test results showed that there were 3 isolates that had the highest antibacterial activity (**Figure 2**), namely SKK 1 MA, SKS 2 ISP, and SKS 4 ISP isolates. At 24-h observation, isolates SKK 1 MA, SKS 2 ISP, and SKS 4 ISP had inhibition zones of 3.5±0.7, 6.5±0.7, 12±0 mm against *S. aureus*, while the inhibition zones formed against *E. coli* were SKK 1 MA (4±0 mm), SKS 2 ISP (12±0 mm), SKS 4 ISP (12±0 mm), and the inhibition zones formed against *S. typhi* were SKK 1 MA (1 ±1.41 mm), SKS 2 ISP (4±0 mm), and SKS 4 ISP (7.5±0.7 mm). At 48 h of observation, the 3 isolates experienced an increase in the area of the inhibition zone with SKK 1 MA (3.5±0.7 mm), SKS 2 ISP (11.5±0.7 mm), and SKS 4 ISP (14±0 mm) against *S. aureus*, SKK 1 MA (4±0 mm), SKS 2 ISP (12±0 mm), and SKS 4 ISP (12±0 mm) against *E. coli*, and SKK 1 MA (1.5±2.12 mm), SKS 2 ISP (7 ±0 mm), and SKS 4 ISP (9.5±3.53 mm) against *S. typhi*. According to Khasanah and Nastiti [23], the presence of a clear zone around the paper disk and an increase in the area of the inhibition zone indicated that the 3 isolates had bactericidal -antifungal activity. According to Erlyn [24], the level of bacterial inhibitory power was categorized into very strong (> 20 mm), strong (10 - 20 mm), moderate (5 - 10 mm), and weak (< 5 mm). The isolates SKK 1 MA, SKS 2 ISP, and SKS 4 ISP had an inhibition zone of about 5 mm-10 mm, which indicated that the 3isolates had moderate antibacterial activity.

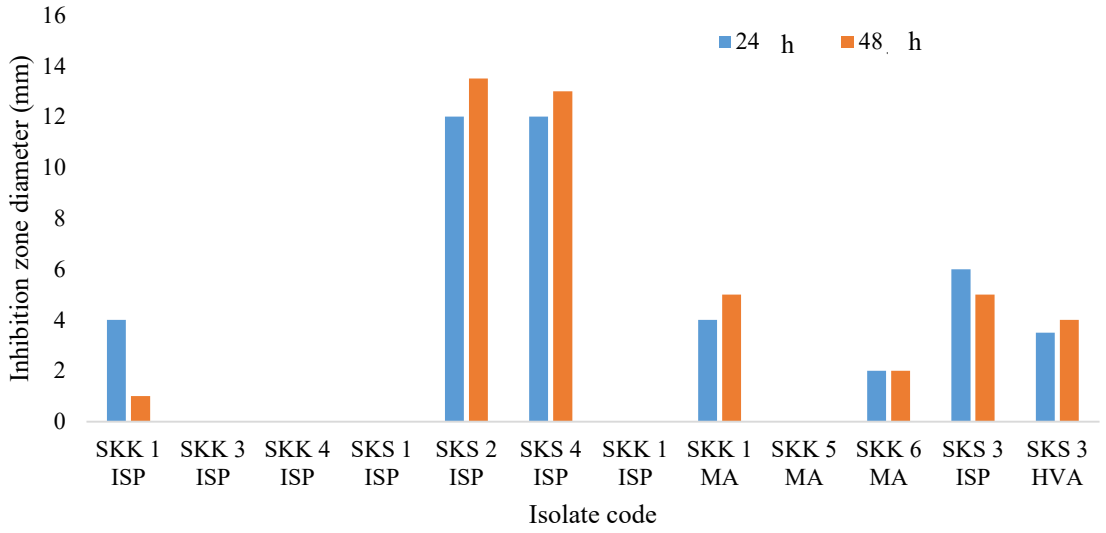

Figure 2 Antibacterial test results of mangrove sediment Isolates against *E. coli.*

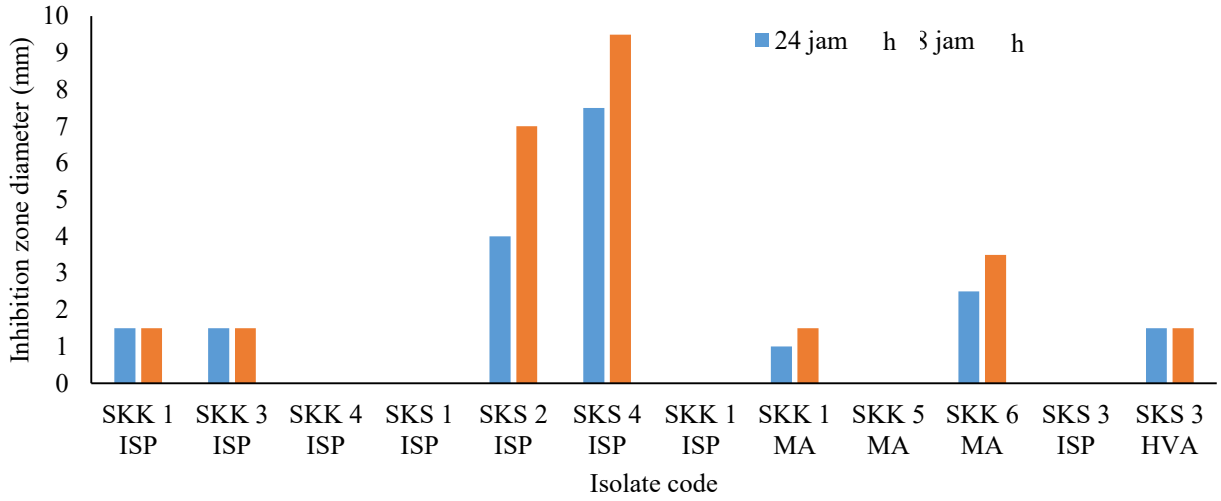

Figure 3 Antibacterial Test Results of Mangrove Sediment Isolates against *S. typhi.*

Molecular identification of isolates

Results of the molecular identification of isolates can be seen in the following **Table 3**.

Table 3 Results of the molecular identification of mangrove sediment isolate species.

Code	Relative similarity	Query cover	E value	Percent identify	Acc number
SKK 1 MA	*Bacillus sp.*	99 %	0	99.47 %	AY986796.1
SKS 2 ISP	*Bacillus cereus*	100 %	0	99.50 %	MN148884.1
SKS 4 ISP	*Alcaligenes faecalis*	100 %	0	100 %	MN493920.1

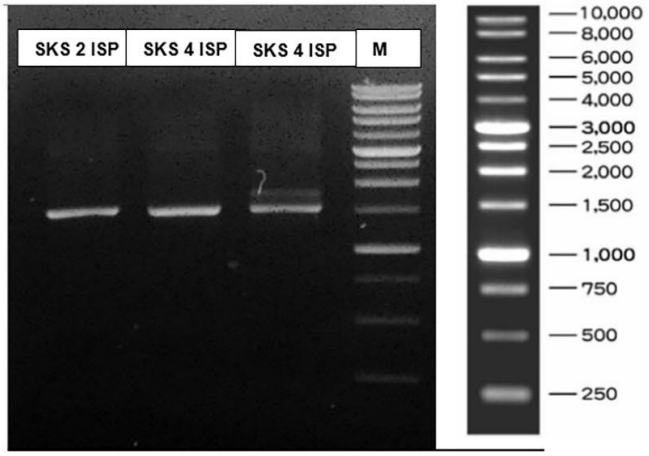

Figure 4 DNA amplification results of SKK 1 MA, SKS 2 ISP, and SKS 4 ISP isolates.

GC-MS analysis

The analysis results of the bacterial content found, namely the bacteria *Bacillus sp*. (SKK 1 MA isolate), showed that the highest content was Octadec-9-Enoic acid (28.60 %), then n-Hexadecanoic acid (27.64 %) and Hexadecanoic acid, methyl ester (CAS) (15.94 %). Meanwhile, the bacterial isolate *Bacillus cereus* (SKS 2 ISP) showed that the highest content was 9-Octadecenoic acid (Z) methyl ester. C16H32O2 (45.11 %), Octadecanoic acid, C19H36O2 (23.86 %) and Tetracosanoic acid, methyl ester (CAS), C25H50O2 (18.60 %). Meanwhile, the bacteria *Alcaligenes faecalis* (SKS 4 ISP) showed that the highest content was n-Hexadecanoic acid, C16H32O2 (33.05 %), Octadec-9-enoic acid, C18H34O2 (30.26 %) as shown in **Tables 4 - 6**.

Table 4 GC-MS analysis results of isolate SKK 1 MA.

Peak#	R.Time	Area	Area%	Height	Name	Formula	MolWeight
1	3.020	1437829	0.40	2088993	2-Methyl-2-(alpha-thienyl)-1,3-dithiolane	C8H10S3	202
2	18.395	3695659	1.02	1105093	PENTADECANE	C15H32	212
3	28.487	57813808	15.94	12865337	Hexadecanoic acid, methyl ester (CAS)	C17H34O2	270
4	30.042	100259046	27.64	13311646	n-Hexadecanoic acid	C16H32O2	256
5	30.275	8042883	2.22	1281107	Cyclopropaneoctanoic acid, 2-hexyl-, methyl ester (CAS)	C18H34O2	282
6	32.034	40691883	11.22	9074345	9-Octadecenoic acid, methyl ester, (E)-	C19H36O2	296
7	32.152	21508489	5.93	3980291	9-Octadecenoic acid, methyl ester, (E)-	C19H36O2	296
8	32.523	9520230	2.62	2181473	Octadecanoic acid, methyl ester	C19H38O2	298
9	33.484	103749746	28.60	8396846	OCTADEC-9-ENOIC ACID	C19H38O2	298
10	33.838	16067212	4.43	2202892	9-Octadecenoic acid (Z)- (CAS)	C18H34O2	282
		362786785	100.0	56488023			

Table 5 GC-MS Analysis Results of Isolate SKS 2 ISP.

Peak#	R.Time	Area	Area%	Height	Name	Formula	MolWeight
1	28.604	978251955	18.60	133571044	Tetracosanoic acid, methyl ester (CAS)	C25H50O2	382
2	30.531	2378478548	45.22	118712726	Hexadecanoic acid (CAS)	C16H32O2	256
3	32.150	1254745949	23.86	160503738	9-Octadecenoic acid (Z)-, methyl ester (CAS)	C19H36O2	296
4	32.565	156577099	2.98	33003056	Octadecanoic acid, methyl ester (CAS)	C19H38O2	298
5	33.588	283185763	5.38	26814004	Oleic Acid	C18H34O2	282
6	34.149	48636099	0.92	9821517	Hexadecanamide (CAS)	C16H33NO	255
7	35.028	36563576	0.70	7441220	Palmitoyl chloride	C16H31ClO	274
8	35.782	38023447	0.72	7162514	Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester (CAS)	C35H68O5	568
9	38.863	44050944	0.84	5261148	DI-(9-OCTADECENOYL)-GLYCEROL	C39H72O5	620
10	39.308	40829927	0.78	6076428	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (CAS)	C19H38O4	330
		5259343307	100.00	508367395			

Table 6 GC-MS analysis results of isolate SKS 4 ISP.

Area%	Height	Name	Formula	MolWeight
7.43	2665492	Dodecanoic acid	C12H24O2	200
2.95	1355051	1-Hexadecene (CAS)	C16H32	224
4.51	1856056	Pentacosanoic acid, 4-methyl-, methyl ester (CAS)	C27H54O2	410
7.17	4973292	Hexadecanoic acid, methyl ester (CAS)	C17H34O2	270
33.05	13253140	n-Hexadecanoic acid	C16H32O2	256
1.75	947564	CIS-10-HEPTADECENOIC ACID ME	C18H34O2	280
5.81	3802814	9-Octadecenoic acid, methyl ester, (E)-	C19H36O2	296
2.36	1349640	9-Octadecenoic acid, methyl ester, (E)- (CAS)	C19H36O2	296
30.26	7503245	OCTADEC-9-ENOIC ACID	C18H34O2	282
4.71	1855581	Octadecanoic acid	C18H36O2	284
100.00	39561875			

GC-MS analysis results of isolate SKS 2 ISP

The analysis results of the bacterial content found, namely the bacteria *Bacillus sp*. (SKK 1 MA), *Bacillus cereus* (SKS 2 ISP), and *Alcaligenes faecalis* (SKS 4 ISP) isolates, showed that bacterial isolates contained saturated and unsaturated fatty acids. The bacteria found have the potential as anti-bacterial and antifungal.

The bacteria found contained saturated fatty acids such as octadecanoic acid, which is a saturated fatty acid that can be easily obtained from animal fat and cooking oil. It is a solid at room temperature, with the chemical formula $CH_3(CH_2)_{16}COOH$. The word stearate comes from the Greek "stear", which means "solid fat". Stearic acid is processed by treating animal fat with water at high temperature and high pressure. This acid can also be obtained from the hydrogenation of vegetable oils. In the industrial sector, stearic acid is used as an ingredient in making candles, soaps, plastics, cosmetics, and softening rubber. The melting point of stearic acid is 69.6 °C and the boiling point is 361 °C. The reduction of stearic acid produces stearyl alcohol [25]. In contrast to saturated fatty acids, unsaturated fatty acids have many health benefits and are found in 3 mangrove sediment bacteria which contain omega 9. Unsaturated fatty acids have double bonds and are liquid at room temperature.

There were 3 types of bacteria found from mangrove sediment isolates that have potential as antibacterial and antifungal agents, namely *Bacillus sp*. *Bacillus cereus* and *Alcaligenes faecalis*. These 3 types of bacteria are known to contain fatty acids. Fatty acids have long chains and are non-polar. Meanwhile, the bacterial membrane is non-polar because it is composed of a double layer of fatty acids, making it affects the lysis process by the fatty acid content. This content also has the potential as an antibacterial and antifungal found in these bacteria.

Bacillus sp. is known as a gram-positive bacteria and considered to analyse the antibacterial activity. This bacteria has potential to produce important enzymes, few of them reported as an alginate lyase producer [26]. Meanwhile *Bacillus cereus* is bacteria that can live in any situation of environment, this bacteria could contaminate by decompositioning and deteriorationing of fish product. The fish that contamined [27], *Bacillus cereus* could be found in seaweed species (*Eucheuma spinosum, Gracilaria gracilis,* and *Glacilaria verrucosa*) [28] and *Bacillus oceanisediminis* could be found in *Holothuria atra* [29].

A. faecalis is a Gram-negative bacterium which appears rod-shaped and motile under a microscope. It is positive by the oxidase test and catalase test, but negative by the nitrate reductase test. It is alpha-hemolytic and requires oxygen. *A. faecalis* can be grown at 37 °C, and forms colonies that lack pigmentation. *A. faecalis* has been used for the production of nonstandard amino acids [30]. The results showed that three bacteria were found, namely *Bacillus sp. Bacillus cereus* and *Alcaligenes faecalis* have antifungal and antibacterial activity. Information from the results of this study is useful in the health sector such as suppressing infectious diseases caused by pathogens and preventing food poisoning caused by bacteria. The research results can also be used as a microorganism agent to eliminate decay processes that threaten human health. The efficacy of some microbial agents currently used for preservatives to improve the safety of food products in the food industry and inhibit disease-causing microorganisms in medicine can also be used in cosmetics [31]. Therefore, the bacteria resulting from this study have great potential to be developed as agents. New and safe antimicrobial. This review summarizes scientific studies on antibacterial and antifungal activity in mangrove sediments from Karimunjawa waters and Semarang waters, Indonesia

Conclusions

There were 17 isolates from Karimunjawa mangrove sediments and 12 isolates from Semarang mangrove sediments. Results of the study concluded that from the isolation of mangrove sediment bacteria from Karimunjawa and Semarang, 3 isolates were obtained, namely isolates SKK 1 MA, SKS 2 ISP, and SKS 4 ISP, which had antibacterial activity against pathogenic bacteria *E. coli*, *S. aureus*, and *S. typhi*. The 3 isolates also had antifungal activity against the fungal pathogens *M. furfur* and *C. albicans*. Molecular identification showed that the 3 potential isolates, namely SKK 1 MA, SKS 2 ISP, and SKS 4 ISP, were from *Bacillus sp*., *Bacillus cereus*, and *Alcaligenes faecalis*, respectively. These bacteria contain saturated fatty acids and unsaturated fatty acids. The research results are promising for information as an interesting material used in the industrial field.

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References

[1] D Ariyanto. Food preference on *Telescopium telescopium* (Mollusca: Gastropoda) based on food sources in mangrove. *Plant Arch.* 2019; **19**, 913-6.

[2] D Ariyanto, DG Bengen, T Prartono and Y Wardiatno. Distribution and abundance of *Cerithideopsilla djadjariensis* (Martin 1899) (Potamididae) on *Avicennia marina* in Rembang, Central Java, Indonesia. *Egypt. J. Aquat. Biol. Fish.* 2020; **24**, 323-32.

[3] D Pringgenies, WA Setyati, A Djunaedi, R Pramesti, S Rudiyanti and D Ariyanto. Exploration of antimicrobial potency of mangrove symbiont against multi-drug resistant bacteria. *Jurnal Ilmiah Perikanan dan Kelautan* 2021; **12**, 222-32.

[4] D Ariyanto, DG. Bengen, T Prartono and Y Wardiatno. Short communication: The relationship between content of particular metabolites of fallen mangrove leaves and the rate at which the leaves decompose over time. *Biodiversitas* 2018; **19**, 780-5.

[5] D Ariyanto, H Gunawan, D Puspitasari, SS Ningsih, A Jayanegara and H Hamim. Identification of the chemical profile of *Rhizophora mucronata* mangrove green leaves from the eastern coast of Asahan, North Sumatra, Indonesia. from Asahan Regency, North Sumatra, Indonesia. *Plant Arch.* **19**, 4045-9.

[6] A Engering, L Hogerwerf and J Slingenbergh. Pathogen-host-environment interplay and disease emergence. *Emerg. Microb. Infect.* 2013; **2**, 1-7.

[7] SR Parratt and A Laine. Pathogen dynamics under both bottom-up host resistance and top-down hyperparasite attack *J. Appl. Ecol.* 2018; **55**, 2976-85.

[8] MR. Hilleman. Strategies and mechanisms for host and pathogen survival in acute and persistent viral infections. *Proc. Natl. Acad. Sci.* 2004; **101**, 14560-6.

[9] P Sharma and D Thakur. Antimicrobial biosynthetic potential and diversity of culturable soil actinobacteria from forest ecosystems of Northeast India. *Sci. Rep.* 2020; **10**, 4104.

[10] P Carbonell, A Currin, AJ Jervis, NJW Rattray, N Swainston, C Yan, ETakano, R Breitling. Bioinformatics for the synthetic biology of natural products: Integrating across the Design-Build-Test cycle. *Nat. Prod. Rep.* 2016; **33**, 925-32.

[11] D Pringgenies and WA Setyati. Antifungal strains and gene mapping of secondary metabolites in mangrove sediments from Semarang city and Karimunjawa islands, Indonesia. *AIMS Microbiol.* 2021; **7**, 499-512.

[12] WA Setyati, D Pringgenies, N Soenardjo and R Pramesti. Actinomycetes of secondary metabolite producers from mangrove sediments, Central Java, Indonesia. *Vet. World* 2021; **14**, 2620-4.

[13] ON Sekurova, O Schneider and SB Zotchev. Novel bioactive natural products from bacteria via bioprospecting, genome mining and metabolic engineering. *Microb. Biotechnol.* 2019; **12**, 828-44.

[14] OF Davies-Bolorunduro, IA Adeleye, MO Akinleye and PG Wang. Anticancer potential of metabolic compounds from marine actinomycetes isolated from Lagos Lagoon sediment. *J. Pharm. Anal.* 2019; **9**, 201-8.

[15] Q Li, X Chen, Y Jiang and C Jiang. *Morphological identification of actinobacteria*. IntechOpen, London, 2016, p. 59-86.

[16] M Hayakawa and H Nonomura. Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. *J. Ferment. Tech.* 1987; **65**, 501-9.

[17] A Margarida, M Olívia and A Lourenço. MorphoCol: An ontology-based knowledgebase for the characterisation of clinically significant bacterial colony morphologies. *J. Biomed. Inform.* 2015; **55**, 55-63.

[18] O Messaoudi and J Wink. Diversity of actinobacteria isolated from date palms rhizosphere and saline environments: Isolation , identification and biological activity evaluation. *Microorganisms* 2020; **8**, 1853.

[19] WA Setyati, E Martani and M Zainuddin. Consortium of mangrove ecosystems as bioremediation and biocontrol in shrimp ponds. *Jurnal Pengolahan Hasil Perikanan Indonesia* 2015; **17**, 243-53.

[20] R Kristiana, G Bedoux, G Pals, IM Wayan, L Taupin, C Marty, MA Asagabaldan, D Ayuningrum, A Trianto, N Bourgougnon, OK Radjasa, A Sabdono and M Hanafi. Bioactivity of compounds secreted by symbiont bacteria of Nudibranchs from Indonesia. *PeerJ* 2020; **1**, e8093.

[21] M El Samak, SM Solyman and A Hanora. Antimicrobial activity of bacteria isolated from Red Sea marine invertebrates. *Biotechnol. Rep.* 2018; **19**, e00275.
[22] NF Hasanah, D Pringgenies and SY Wulandari. Characterization of *Conus miles* gastropod symbion bacteria secondary metabolites using the GC-MS method as MDR antibacterial (multi drug resistant). *J. Mar. Res.* 2012; **141**, 548-9.
[23] AU Khasanah and SJ Nastiti. Identification of the active compound of tobacco leaf extract (*Nicotiana tabacum* L.) as antibacterial against *Staphylococcus aureus. Al-Hayat J. Biol. Appl. Biol.* 2021; **4**, 19-32.
[24] P Erlyn. Antibacterial effectiveness of the active fraction of lemongrass (*Cymbopogon citratus*) against *Streptococcus mutans*. *Syifa'MEDIKA* 2016; **6**, 111-25.
[25] R Rodiansono, E Hayati, AS Azzahra, MD Astuti, K Mustikasari, S Husain and S Sutomo. Selective hydrogenation of stearic acid to 1-octadecanol using bimetallic palladium-tin supported on carbon catalysts at mild reaction conditions. *Bull. Chem. React. Eng. Catal.* 2021; **16**, 888-903.
[26] DS Zilda, Y Yulianti, RF Sholihah, S Subaryono, YN Fawzya and HE Irianto. A novel *bacillus sp.* Isolated from rotten seaweed: Identification and characterization alginate lyase its produced. *Biodiversitas* 2019; **20**, 1166-72.
[27] J Olmos, M Acosta, G Mendoza and V Pitones. *Bacillus subtilis*, an ideal probiotic bacterium to shrimp and fish aquaculture that increase feed digestibility, prevent microbial diseases , and avoid water pollution. *Arch. Microbiol.* 2020; **202**,427-35.
[28] D Pringgenies, IE Retnowati, D Ariyanto, K Dewi, MAS Viharyo and R Susilowati. Symbiotic microbes from various seaweeds with antimicrobial and fermentative properties. *AACL Bioflux* 2020; **13**, 2211-7
[29] GW Santosa, A Djunaedi, A Susanto, D Pringgenies, D Ariyanto. Characteristics of bioactive compounds of *Holothuria atra* (Jaeger, 1833) associated bacteria. *AACL Bioflux* 2020;**13**,2161-9.
[30] B Ashrafian and A Hosseini-Abari. Investigation of bioactivity of unsaturated oligo-galacturonic acids produced from apple waste by *Alcaligenes faecali*s AGS3 and *Paenibacillus polymyxa* S4 Pectinases. *Sci. Rep.* 2022; **12**, 15830.
[31] D Pringgenies, WA Setyati, F Feliatra and D Ariyanto. The antibacterial and antifungal potential of marine natural ingredients from the symbiont bacteria of mangrove. *Global J. Environ. Sci. Manag.* 2023; **9**, 1-14.