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Antibacterial and Enzymatic Activities of Symbiotic Bacteria from Gastropods and Bivalves in Marine Skincare Applications

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ABSTRACT

Marine symbiotic bacteria found in mollusks from the seagrass and coral reef ecosystem have shown potential as skincare products, specifically to treat acne. This study aimed to investigate the antibacterial and enzyme-producing abilities of these bacteria for their potential as natural cosmetic ingredients. Symbiotic bacteria were isolated from bivalves and gastropods collected from Padak Brambang, Lombok, West Nusa Tenggara, Indonesia. The isolates were tested against Cutibacterium acnes, Staphylococcus aureus, and Staphylococcus epidermidis for antibacterial activity using the disc diffusion method, and lipase and protease enzymes were detected. The top isolates, G4LC2.3 and G8LM2.4, showed strong antibacterial activity, while B4KS2.3 and G2LA2.1 demonstrated high enzyme production. Pseudomonas aeruginosa, Vibrio diabolicus, and Vibrio owensii were identified as potential species for use in cosmetics. Analysis of the bacterial compounds revealed potential ingredients such as 1-pyrroline-5-carboxylic acid, succinic acid, and dihydropinosylvin methyl ether in P. aeruginosa, and 5-hydroxy-3-(4-hydroxyphenyl)-4-phenylpyrrol-2-one, fuculose, and 2-hydroxy-5-[(1e,3z,5e,7r,8r,9e)-8-hydroxy-7,9,12-trimethyltrideca-1,3,5,9-tetraen-1-yl]-2,4-dimethylfuran-3-one in *V. diabolicus* and *V. owensii*. These findings suggest that symbiotic bacteria from mollusks can be used as environmentally friendly and effective natural ingredients in marine skincare applications. The study highlighted the potential of these isolates for cosmetic use.

1. INTRODUCTION

The marine ecosystem is home to a vast array of organisms, including microbial organisms such as bacteria. Among them, symbiotic bacteria that live in a mutualistic relationship with their host organisms have received increasing attention for their potential applications in various industries [1]. In particular, mollusks in Lombok's seagrass and coral reef ecosystems have been found to have complex interactions with their bacterial symbionts. These symbiotic bacteria have been identified as a promising source of active compounds that can be beneficial to the cosmetic industry [2]. For instance, the compounds derived from symbiotic bacteria may exhibit antioxidant properties [3] or demonstrate antibacterial effects [4], making them attractive for use in natural skincare products.

One of the main advantages of natural skincare ingredients is that they are typically free from harmful chemicals or dangerous substances. This makes them not only safe for use in various products but also unlikely to cause irritation or allergies that can be harmful to health. Additionally, natural ingredients are rich in nutrients and effective properties, offering similar or even superior benefits compared to synthetic ingredients commonly found in beauty and healthcare products. They are also suitable for all skin types, including sensitive skin, as they are gentle and unlikely to cause irritation. Furthermore, natural ingredients have been proven to address common skin issues such as acne, dry skin, and premature aging [5].

In particular, acne vulgaris is a skin condition that occurs when the facial skin is in poor condition. To address this issue, anti-acne cosmetic products use active compounds that can inhibit the growth of acne-causing bacteria, exfoliate dead skin cells, and control excess sebum production [6]. However, the excessive use of antibiotics in these products can lead to the development of antibiotic-resistant pathogenic bacteria, making acne treatment less effective in the long run. In this respect, compounds derived from symbiotic bacteria found in mollusks and gastropods offer an alternative approach to acne treatment, as they have been found to possess antibacterial properties [7].

Beyond addressing acne, symbiotic bacteria from bivalves and gastropods have also been investigated for their potential to combat skin aging. The aging process is accelerated by

external factors such as exposure to UV radiation, pollution, and stress. The presence of symbiotic bacteria in bivalve and gastropod hosts has been found to maintain the balance of microbes on facial skin and prevent skin damage, which can accelerate the aging process. Moreover, several bioactive compounds produced by these symbiotic bacteria have been shown to have anti-aging properties, such as increasing skin moisture, enhancing collagen production, and reducing wrinkles [8]. Additionally, symbiotic bacteria have been found to contribute to reducing pigmentation on facial skin, commonly caused by excessive melanin production triggered by exposure to UV rays or oxidative stress. The compounds produced by symbiotic bacteria have been found to possess depigmentation properties, which can reduce melanin production and inhibit the pigmentation process on facial skin [9]. These properties make symbiotic bacteria an attractive ingredient for use in whitening products.

Apart from the benefits for facial skin, the use of symbiotic bacteria in cosmetic products has a potential positive impact on the environment. Natural ingredients derived from symbiotic bacteria can reduce exploitation rates and help maintain healthy marine ecosystems, which are rich in biodiversity. This reduces the use of chemicals and antibiotics, which may have negative effects on the environment.

The symbiotic bacteria found in mollusks and gastropods have significant potential in the cosmetics industry. The compounds they produce have been found to offer a variety of benefits for facial skin, such as combating acne and signs of aging and reducing pigmentation. Moreover, their use in cosmetic products can also contribute to the protection of marine biodiversity and the environment. Further research in this area has the potential to unlock the full potential of these bacteria and provide more natural and environmentally friendly options for skincare products.

2. MATERIALS AND METHODS

2.1 Sample colletion

Samples of gastropods and bivalves were collected from the waters of Padak Brambang, Lombok, West Nusa Tenggara Province, Indonesia. The sampling was conducted in seagrass and coral reef ecosystems. Each mollusk sample was identified by its morphological characteristics at the species level [10].

2.2 Isolation, characterization, and purification of bivalve and gastropod symbiont bacteria

Bacteria isolation was conducted using the spread plate method. A total of 1 g samples was dissolved in 1mL of sterile seawater. The solution was diluted to 10^{-4}. Each dilution was inoculated into marine nutrient agar media (OXOID brand nutrient agar dissolved in 100% seawater) and incubated at 28℃ for 3×24 hours [11]. The bacteria that successfully grew on the media were separated and purified using the streak method [7].

2.3 Antibacterial activity

The antibacterial test was performed using Mueller-Hinton Agar (MHA) media and the disc diffusion method. The pathogenic bacteria used in the tests were *Cutibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*. A sterile nutrient broth (NB) solution served as the negative control (-), while a 2% amoxicillin solution in NB was used as the positive control (+). Observations for the antibacterial tests were conducted over 3×24 hours [7]. Calculating the inhibition zone diameter involved subtracting the diameter of the clear zone from the diameter of the paper disc [12].

2.4 Lipolytic and proteolytic enzymatic test

Enzymatic tests for lipase and protease activity were conducted to assess the extracellular enzyme production by bacteria. The tests were performed using the disc diffusion method. Lipase activity was assessed using marine nutrient agar enriched with tween80 and proteolytic activity was tested using marine nutrient agar media enriched with 1% skim milk. The test was incubated at 28℃ and observed for 3×12 hours [2]. The diameter of the hydrolysis zone for each enzymatic test is measured, and the proteolytic index and lipolytic index are calculated [13].

2.5 Target bacterial DNA extraction and 16S rRNA amplification, sequencing and molecular data processing

Bacterial DNA was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research D6005). The 16S rRNA gene was amplified using MyTaq HS Red Mix, 2x (Bioline, BIO-25048) with a thermal cycler. The sequencing results were analyzed using MEGA 11 software. The primary 16S rRNA data was compared using BLAST (Basic Local Alignment Search Tool) from NCBI GenBank (http://www.ncbi.nlm.nih.gov).

2.6 Liquid Chromatography-Mass Spectrometry (LC-MS) analysis

LC-MS was used to analyze untargeted compounds in the bacteria's crude extract. The raw data obtained was interpreted using MassLynx^{TM} v4.1 software, which interprets the results of the data elemental composition [14].

3. RESULT

The 16 of mollusk samples (gastropods and bivalves), 147 bacterial isolates were obtained. A total of 147 bacterial isolates whose quality was tested against types of pathogenic bacteria, namely *C. acnes, S. aureus,* and *S. epidermidis*, obtained 9 isolates that had potential as antibacterials for these 3 types of pathogenic bacteria. In addition, 9 antibacterial active isolates were quantitatively tested against 3 pathogenic bacteria.

3.1 Antibacterial test

Antibacterial tests were conducted on bacteria found in bivalves and gastropods to evaluate their efectiveness against the pathogenic bacteria *C. acnes, S. aureus*, and *S. epidermidis* (Figure 1). The research findings revealed that isolate code G8LM2.4 displayed bactericidal properties, with an increased antibacterial zone of inhibition rising from 3.84 to 4.23 against the *C. acnes* pathogen. In contrast, isolate G4LC2.3 demonstrated bactericidal effects (measured in millimeters) against the pathogenic bacteria *C. acnes* (from 3.13 to 5.28)

and *S. aureus* bacteria (from 6.48 to 8.08) as depicted in Table 1.

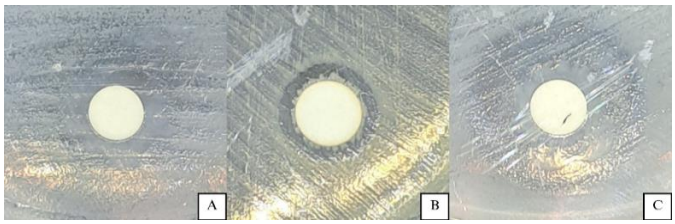

Figure 1. Symbiont bacteria antibacterial test against (A) *C. acnes*, (B) *S. aureus*, and (C) *S. epidermidis*

The antibacterial test results for the G4LC2.3 isolate indicated that it had the largest clear zone of inhibition for both *S. aureus* and *S. epidermidis* pathogens at every hour of observation. This isolate exhibited bactericidal properties against the *S. aureus* pathogen, as evidenced by an increasing diameter of the inhibition zone over time (6.48 ± 0.51 mm at 24 hours, 7.65 ± 1.06 mm at 48 hours, and 8.08 ± 0.59 mm at 72 hours). The same isolate has bacteriostatic properties against the pathogen *S. epidermidis*. This was demonstrated by test results showing an initial diameter of 13.16 ± 0.99 mm after 24 hours of observation. Subsequently, a decrease in diameter was observed at 48 hours (12.13 ± 1.88 mm) and 72 hours (10.64 ± 1.60 mm). An increase or stability in the diameter of the inhibition zone around the test isolate indicates that the isolate has bactericidal properties.

The results revealed that only 9 out of 147 isolates exhibited antibacterial properties against *C. acnes*, *S. aureus*, and *S. epidermidis*. This was attributed to variations in the isolates' ability to combat pathogens, as evidenced by the formation of inhibition zones.

Table 1. Symbiont antibacterial test against pathogenic bacteria

Isolate	Inhibition Zone Diameter (mm)								
	C. acnes			*S. aureus*			*S. epidermidis*		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
(-) Control	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0
(+) Control	22.37±1.81	22.85±0.92	22.54±0.06	17.52±0.21	13.95±1.2	14.4±0.85	22.56±0.04	25.64±0.52	21.11±0.08
B8KP2.2	0.15±0.21	3.28±0.86	4±1.46	18.25±1.77	7.03±2.16	6.54±0.76	-	-	-
G4LC2.3	3.13±0.82	4.43±1.95	5.28±1.32	6.48±0.51	7.65±1.06	8.08±0.59	13.16±0.99	12.13±1.88	10.64±1.6
G8LM2.4	3.84±0.16	3.92±0.14	4.23±0.27	-	-	-	-	-	-
B1LA4.4	-	-	-	-	-	-	2.76±2.98	2.76±2.63	3.06±2.09
G8LM2.1	-	-	-	-	-	-	2.18±0.34	1.61±1.57	0.72±0.45
G6LS2.2	-	-	-	-	-	-	8.63±4.79	14.61±3.83	1.59±0.83
G6LS4.3	-	-	-	-	-	-	8.45±3.14	9.5±0.71	1.32±1.44
B2KM2.2	-	-	-	-	-	-	3.1±2.67	2.96±2.47	3.36±1.26
G5LA2.1	-	-	-	-	-	-	2.75±3.65	2.75±3.32	2.75±3.18

*Inhibition zone (mean±SD)

Table 2. Enzymatic activity test of symbiotic bacteria

Isolate	Lipolytic Index			Proteolytic Index		
	12 h	24 h	36 h	12 h	24 h	36 h
(-) Control	0±0	0±0	0±0	0±0	0±0	0±0
B1KH4.2	0±0	0±0	0±0	0.13±0.04	0.45±0.24	0.78±0.40
B1LA4.2	0.85±0.14	1.46±0.17	2.43±0.20	0.81±0.04	0.85±0.22	0.83±0.46
B1LA4.3	0±0	0±0	0±0	0.31±0.15	0.72±0.22	0.64±0.27
B3KO2.1	0.40±0.02	1.70±0.18	3.48±0.14	0.12±0.07	0.25±0.08	0.68±0.2
B4KS2.2	0±0	0±0	0±0	0.40±0.17	0.24±0.07	0.67±0.39
B4KS2.3	1.04±0.17	5.03±0.10	7.92±0.40	0±0	0±0	0±0
B4KS4.2	0.19±0.08	4.61±0.29	6.93±0.25	0.06±0.16	0.50±0.09	0.62±0.24
B5KO4.5	0±0	0.64±0.11	0.55±0.12	0.23±0.13	0.62±0.17	0.62±0.07
B6KC2.1	0±0	0±0	0±0	0.25±0.23	0.36±0.27	0.34±0.23
B6KC2.4	1.01±0.04	3.80±0.03	6.61±0.32	0.14±0.08	0.44±0.23	0.61±0.23
B8KP2.1	0.47±0.09	2.72±0.48	3.45±0.17	0.39±0.13	0.93±0.06	0.77±0.07
G2LA2.1	0±0	0±0	0±0	0.91±0.08	1.24±0.08	1.77±0.09
G4LC2.2	0±0	1.06±2.91	2.25±4.60	0±0	0±0	0±0
G4LC4.1	1.64±0.29	6.67±0.06	6.63±0.37	0±0	0±0	0±0
G5LA2.4	0±0	2.43±0.11	3.30±0.10	0±0	0±0	0±0
G5LA4.1	0.27±0.04	4.45±0.03	6.28±0.10	0.31±0.13	0.69±0.05	0.45±0.35
G6LS4.1	0±0	0±0	0±0	0.1±0.18	0.11±0.29	0.76±0.22
G6LS4.2	0±0	0±0	0±0	0.31±0.11	0.28±0.02	0.62±0.13
G7CM2.6	0.12±0.09	2.99±0.06	6.88±0.12	0.29±0.01	0.06±0.02	0.35±0.05
G11LC2.1	0.35±0.09	5.46±0.37	7.18±0.54	0.28±0.06	1.58±0.03	0.15±0.15

*Results of Enzymatic Index (mean±SD)

3.2 Lipolytic and proteolytic enzymatic test

Quantitative enzymatic testing using the disc diffusion method by symbiotic bacteria revealed that 13 isolates exhibited lipolytic enzymatic activity, and 17 isolates demonstrated proteolytic enzymatic ability (Figure 2). Isolates B1LA4.2, B3KO2.1, B4KS4.2, B5KO4.5, B6KC2.4, B8KP2.1, G5LA4.1, and G11LC2.1 were found to have the capability to produce both lipase and protease enzymes. The isolate with the highest lipolytic ability at the final hour of

observation was B4KS2.3, while the one with the highest proteolytic ability was G2LA2.1, as shown in Table 2.

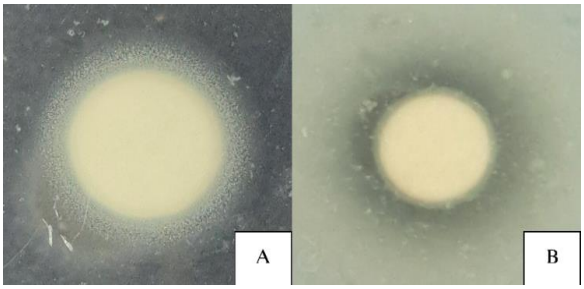

Figure 2. Symbiont bacteria (A) lipolytic test and (B) proteolytic test

3.3 Molecular identification

Molecular identification identified 4 potential isolates in marine mollusks, namely *Pseudomonas aeruginosa* (100%) (G4LC2.3), *Pseudomonas aeruginosa* (100%) (G8LM2.4), *Vibrio diabolicus* (99.78%) (B4KS2.3), and *Vibrio owensii* (100%) (G2LA2.1). The results showed each identified isolate was characterized by 1360-1414bp. This range is in accordance with bacteria nucleotide length general characteristics (~1400bp). The molecular identification results are shown in Table 3.

3.4 LC-MS analysis

LC-MS analysis was conducted on crude extracts of specific bacteria isolates (G4LC2.3, G8LM2.4, B4KS2.3, and G2LA2.1) to separate, predicted, and quantify the compounds present in the samples. The analysis revealed approximately 19-21 peaks in each sample. Additionally, the LC-MS results of *P. aeruginosa* isolates with codes G4LC2.3 and G8LM2.4 predicted that the predominant compounds, based on area percentage, were 1-pyrroline-5-carboxylic acid (15.9% for G4LC2.3 and 15.2% for G8LM2.4), 3-methoxy-5-phenethylphenol (dihydropinosylvin methyl ether) (14.5% for G4LC2.3 and 16.9% for G8LM2.4), and succinic acid (11.5% for G4LC2.3 and G8LM2.4). The LC-MS analysis results are shown in Table 4.

Table 3. Molecular identification results of selected symbiont bacteria

Isolate	Host Species	Relative Similarity	Percent Similarity	Query Cover	Acc Number
G4LC2.3	*Conus magus* (Linnaeus, 1758)	*Pseudomonas aeruginosa*	100%	100%	MT337602.1
G8LM2.4	*Monetaria moneta* (Linnaeus, 1758)	*Pseudomonas aeruginosa*	100%	100%	MT337602.1
B4KS2.3	*Spondylus squamosus* (Schreibers, 1793)	*Vibrio diabolicus*	99.78%	100%	MT020414.1
G2LA2.1	*Canarium urceus* (Linnaeus, 1758)	*Vibrio owensii*	100%	100%	MT510178.1

Table 4. LC-MS analysis of *Pseudomonas aeruginosa* crude extract (G4LC2.3 and G8LM2.4)

R.T (min)	% Area	Compound Name	Molecular Weight (g/mol)
		***P. aeruginosa* (100%) (G4LC2.3)**	
0.30	15.9	1-pyrroline-5-carboxylic acid	113.11
0.54	3.9	Acetylpyruvic acid	130.10
0.76	11.5	Succinic acid	118.09
1.53	4.9	N-acetyl-L-2-aminoadipic acid	203.19
3.54	1.7	2-aminomuconic 6-semialdehyde	141.12
4.08	3.7	N-Methoxyspirobrassinol	282.38
4.38	1.6	Styrolide A	216.23
4.50	6.6	N-[(2R,3E)-5-Hydroxy-4-methyl-3-penten-2-yl]adenosine	365.39
4.67	1.3	Maculosin	260.29
4.79	6.9	(5-Methyl-4-pentadec-7-enyl-2,3-dihydropyrrol-1-yl)-phenylmethanone	395.62
5.04	4.1	Anhydrotetrodotoxin	301.25
5.24	2.7	Pseudotetratide A	527.66
6.37	2.3	Lahorenoic acids C	244.33
6.51	1.5	Eriodictiol	288.26
6.59	1.0	Lahorenoic acids A	260.33
7.92	14.5	Dihydropinosylvin methyl ether	228.29
9.25	5.5	Monic acid A	344.40
9.88	4.9	5,7-dimethoxyphenanthrene-2,3-diol	270.28
10.43	5.7	Methyl 2,3,4-trimethoxyphenazine-1-carboxylate	328.32
		***P. aeruginosa* (100%) (G8LM2.4)**	
0.30	15.2	1-pyrroline-5-carboxylic acid	113.11
0.52	4.2	Acetylpyruvic acid	130.10
0.78	11.5	Succinic acid	118.09
1.52	4.5	N-acetyl-L-2-aminoadipic acid	203.19
4.08	3.2	N-Methoxyspirobrassinol	282.38
4.38	1.3	Styrolide A	216.23
4.50	6.7	N-[(2R,3E)-5-Hydroxy-4-methyl-3-penten-2-yl]adenosine	365.39
4.67	1.5	Maculosin	260.29
4.80	7.2	(5-Methyl-4-pentadec-7-enyl-2,3-dihydropyrrol-1-yl)-phenylmethanone	395.62
5.04	4.0	Anhydrotetrodotoxin	301.25
5.24	2.0	Pseudotetratide A	527.66

6.37	2.6	Lahorenoic acids C	244.33
6.51	1.5	Eriodictyol	288.26
6.61	1.7	Lahorenoic acids A	260.33
7.92	16.9	Dihydropinosylvin methyl ether	228.29
9.27	6.4	Monic acid A	344.40
9.86	5.0	5,7-dimethoxyphenanthrene-2,3-diol	270.28
10.64	3.7	Methyl 2,3,4-trimethoxyphenazine-1-carboxylate	328.32
11.77	3.1	Ricinoleic acid	298.46
12.31	2.5	Pyochelin	324.42

Table 5. LC-MS analysis of *V. diabolicus* (B4KS2.3) and *V. owensii* (G2LA2.1) crude extract

R.T (min)	% Area	Compound Name	Molecular Weight (g/mol)
		***V. diabolicus* (99.78%) (B4KS2.3)**	
0.51	11.34	5-hydroxy-3-(4-hydroxyphenyl)-4-phenylpyrrol-2-one	265.26
0.78	14.13	Fuculose	164.15
1.50	9.60	2-phosphoglyceric acid	186.06
2.39	4.68	3-hydroxy-n-[(3s)-2-oxooxolan-3-yl]hexanimidic acid	215.25
3.22	5.80	Vulnibactin	673.71
4.13	4.18	Solonamides B	586.76
4.55	7.22	Rubrifacine	338.22
4.85	7.00	Xenortide C	395.53
5.83	1.32	Sperabillin B	339.43
6.39	2.65	3-benzyl-1-hydroxy-3h,6h,7h,8h,8ah-pyrrolo[1,2-a]pyrazin-4-one	244.29
6.52	1.73	Psoracorylifol C	288.38
6.62	1.46	3-benzyl-1,7-dihydroxy-3h,6h,7h,8h,8ah-pyrrolo[1,2-a]pyrazin-4-one	260.29
8.11	19.05	2-hydroxy-5-[(1e,3z,5e,7r,8r,9e)-8-hydroxy-7,9,12-trimethyltrideca-1,3,5,9-tetraen-1-yl]-2,4-dimethylfuran-3-one	360.48
8.75	1.12	N-myristoyl-d-asn	342.47
9.03	0.90	Serratamic acid	275.34
9.24	1.07	1,4-dihydroxy-2,5-dimethoxyanthracene-9,10-dione	300.26
10.32	1.16	1-benzoyl-2-methyl-3-tridecyl-4,5-dihydropyrrole	369.58
13.37	2.72	DKxanthene 560	560.64
15.74	2.86	Phytoene	544.93
		***V. owensii* (100%) (G2LA2.1)**	
0.47	23.05	5-hydroxy-3-(4-hydroxyphenyl)-4-phenylpyrrol-2-one	265.26
0.78	16.46	Fuculose	164.15
1.47	8.65	2-phosphoglyceric acid	186.06
2.41	3.82	3-hydroxy-n-[(3s)-2-oxooxolan-3-yl]hexanimidic acid	215.25
2.58	0.35	Dihydroxy-dehydro tilivalline	347.37
3.22	6.67	Vulnibactin	673.71
4.13	8.69	Solonamides B	586.76
4.55	13.90	Rubrifacine	338.22
4.85	10.61	Xenortide C	395.53
5.83	3.18	Sperabillin B	339.43
6.39	3.71	3-benzyl-1-hydroxy-3h,6h,7h,8h,8ah-pyrrolo[1,2-a]pyrazin-4-one	244.29
6.52	2.28	Psoracorylifol C	288.38
6.62	2.36	3-benzyl-1,7-dihydroxy-3h,6h,7h,8h,8ah-pyrrolo[1,2-a]pyrazin-4-one	260.29
7.18	1.48	Butyl acetate	116.15
8.11	21.10	2-hydroxy-5-[(1e,3z,5e,7r,8r,9e)-8-hydroxy-7,9,12-trimethyltrideca-1,3,5,9-tetraen-1-yl]-2,4-dimethylfuran-3-one	360.48
8.75	1.42	N-myristoyl-d-asn	342.47
9.03	1.18	Serratamic acid	275.34
9.25	2.52	1,4-dihydroxy-2,5-dimethoxyanthracene-9,10-dione	300.26
10.32	1.36	1-benzoyl-2-methyl-3-tridecyl-4,5-dihydropyrrole	369.58
13.38	2.54	DKxanthene 560	560.64
15.74	2.45	Phytoene	544.93

The LCMS analysis results of bacterial isolates *V. diabolicus* (B4KS2.3) and *V. owensii* (G2LA2.1) showed that the dominant compound predicted in both isolates was 5-hydroxy-3-(4-hydroxyphenyl)-4-phenylpyrrol-2-one (11,34% for B4KS2.3 and 23,05% for G2LA2.1), fuculose (14,13% for B4KS2.3 and 16,46% for G2LA2.1), and 2-hydroxy-5-[(1e,3z,5e,7r,8r,9e)-8-hydroxy-7,9,12-trimethyltrideca-1,3,5,9-tetraen-1-yl]-2,4-dimethylfuran-3-one (19,05% for B4KS2.3 and 21,10% for G2LA2.1), as shown in Table 5.

4. DISCUSSION

The differences in antibacterial activity in Table 1 can be attributed to the diverse metabolic capabilities of each tested isolate. This is because different bacteria have varying abilities to produce secondary metabolites, which can play a role in their antimicrobial properties [15]. These metabolites, also known as natural products, are produced by bacteria as a form of self-defense to compete with other microorganisms in their environment [16]. They can also help bacteria adapt to

environmental stress, aid in providing nutrition to the host, and attract or repel other organisms [17].

The specific manifestations of metabolic differences can be seen in different strains' secondary metabolite composition and antibacterial spectrum. For example, *Pseudomonas aeruginosa* (G4LC2.3 and G8LM2.4) were found to have potent antibacterial activity against all tested bacteria, while the other isolates showed weaker or no activity against specific strains. This could be due to the distinct composition of secondary metabolites produced by each strain. In fact, numerous secondary metabolites produced by bacteria from marine environments have been discovered to possess potent antimicrobial properties [16]. The metabolic capabilities of bacteria can also be influenced by various factors such as nutrient availability, temperature, and pH, which can result in variations in antimicrobial activity [18].

Further investigation into the biochemical pathways involved in the production of specific compounds can shed light on the diverse metabolic capabilities seen in these symbiotic bacteria. For instance, succinic acid is a dicarboxylic acid produced by *P. aeruginosa*, and its biosynthesis involves a complex network of biochemical pathways. The key enzymes involved in succinic acid production have been identified, including succinate dehydrogenase, fumarase, and malate dehydrogenase. These enzymes are regulated by different factors such as pH, oxygen levels, and nutrient availability, which can impact the yield of succinic acid production [19]. In marine environments, the regulation of biochemical pathways in bacteria can be affected by changes in salinity, temperature, and the presence of other microorganisms, thus influencing the production of important compounds like succinic acid [20, 21].

Similarly, the biosynthesis of 1-pyrroline-5-carboxylic acid in *P. aeruginosa* involves various enzymes and regulatory mechanisms. This compound is a product of the proline conversion cycle, which is activated under stress conditions [22]. This pathway is regulated by the enzyme ornithine cyclodeaminase, which catalyses the conversion of ornithine to proline [23]. This regulatory mechanism allows bacteria to defend against infections caused by various pathogens, highlighting the ecological significance of this compound in marine environments. Additionally, the production of L-proline and L-ornithine by bacteria can also serve as important alternative sources for the synthesis of cosmetics and pharmaceuticals [24].

Table 2 showed that bacteria that naturally produce lipase and protease enzymes play a role in decomposing complex organic compounds in water. Hydrolytic enzymes such as lipase, protease, amylase, and cellulase are involved in breaking down polymers into monomer units in nature [25]. Bacteria produce various enzymes as a form of self-defence to adapt to the environment and provide nutrition to the host [17]. The symbiotic association between microbes and their hosts is aimed at providing important nutrients and high-energy compounds. The lipase and protease enzymes produced by bacteria can serve as new sources for biotechnology applications and the cosmetics industry [26].

A compound known as 5-hydroxy-3-(4-hydroxyphenyl)-4-phenylpyrrol-2-one has a key characteristic of being a potential antioxidant due to the presence of a benzene group [27]. Fuculose is a simple sugar with the chemical formula $C_6H_{12}O_5$, and it is a ketose sugar that is a biosynthesis product of L-fuculose-1-phosphate. Marine bacteria like *Flammeovirga pacifica* can produce enzymes that degrade polysaccharides, producing monosaccharides such as fuculose, which the bacteria can reuse [28]. Another compound with the chemical name 2-hydroxy-5-[(1e,3z,5e,7r,8r,9e)-8-hydroxy-7,9,12-trimethyltrideca-1,3,5,9-tetraen-1-yl]-2,4-dimethylfuran-3-one was found to make up 19.05% of the area in B4KS2.3 and 21.10% of the area in G2LA2.1. This compound contains solid and hashed wedged OH bonds and possesses a carbonyl group that can interact with various non-covalent substances. This interaction is significant as it enables molecules to participate in electrostatic interactions by utilizing positively charged carbon centers and negatively charged oxygen centers [27].

The production of these compounds by symbiotic bacteria in mollusks may play an essential role in the host's defense against pathogens and in maintaining a beneficial symbiotic relationship. The ability of bacteria to produce antimicrobial and beneficial compounds has been shown to be crucial for their survival and adaptation to various environments [29]. In marine environments, bacteria can face various stressors such as changes in temperature, nutrient availability, and predation, which can influence their metabolic capabilities [30]. Thus, bacteria that possess diverse metabolic capabilities may have a better chance of survival and successful symbiosis with their hosts.

The diverse metabolic capabilities of symbiotic bacteria from gastropods and bivalves in the marine environment have been shown to have potential applications as natural cosmetic ingredients. The different antibacterial and enzyme-producing abilities of these is environmentally friendly isolates illustrate their potential for use in marine skincare products. Furthermore, the identification of specific compounds produced by these bacteria, such as succinic acid, 1-pyrroline-5-carboxylic acid, and fuculose, highlights their potential as ingredients in the cosmetics industry. However, further research is needed to fully understand the biochemical pathways involved and the regulatory mechanisms of these compounds in bacteria. This will open up opportunities for the development of new, environmentally-friendly cosmetics and pharmaceuticals based on the diverse metabolic capabilities of symbiotic bacteria from marine environments.

5. CONCLUSIONS

The results of this study suggest that symbiotic bacteria from marine mollusks have the potential to be used as natural ingredients in skincare products. The isolated bacteria showed strong antibacterial activity against *C. acnes, S. aureus*, and *S. epidermidis*, which are known to cause acne. These active bacteria were identified as *P. aeruginosa*, a species commonly found in the marine ecosystem. The analysis of bacterial compounds revealed the presence of potential ingredients, such as 1-pyrroline-5-carboxylic acid, succinic acid, and dihydropinosylvin methyl ether, which have the potential to act as antibacterials, antioxidants, and enzymes in skincare products. Further, the lipase and protease enzymes produced by the symbiotic bacteria can be used in the development of new pharmaceuticals or cosmetic products. Lipase enzymes can break down oils and fats, making them useful for treating oily skin and acne. Protease enzymes can break down proteins, which can help in exfoliating dead skin cells and promoting skin renewal. The identified *Vibrio* species, *V. diabolicus* and *V. owensii*, have the potential to produce compounds such as 5-hydroxy-3-(4-hydroxyphenyl)-4-phenylpyrrole-2-one,

fuculose, and 2-hydroxy-5-[(1e,3z,5e,7r,8r,9e)-8-hydroxy-7,9,12-trimethyltride-1,3,5,9-tetraen-1-yl]-2,4-dimethylfuran-3-one, which have proven to be beneficial in natural bioproducts. These compounds have antioxidant and antibacterial properties that make them suitable for skincare applications. Overall, the findings of this study highlight the potential of symbiotic bacteria from mollusks as environmentally friendly and effective natural ingredients in marine skincare applications. Further research and development are needed to fully utilize the potential of these bacteria and their compounds in skincare products.

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REFERENCES

[1] Pringgenies, D., Soenardjo, N., Setyati, W.A., Pramesti, R. (2024). Secondary metabolites from symbiotic bacteria of seagrass and molluscs as a reference for natural food preservatives. Trends in Sciences, 21(5): 7404. https://doi.org/10.48048/tis.2024.7404

[2] Setyati, W.A., Pringgenies, D., Soenardjo, N., Pramesti, R. (2023). Enzyme-producing symbiotic bacteria in gastropods and bivalves molluscs: Candidates for bioindustry materials. Biodiversitas, 24(1): 20-25. https://doi.org/10.13057/biodiv/d240103

[3] Kusmita, L., Edi, A.N.P., Franyoto, Y.D., Mutmainah, Haryanti, S., Nurcahyanti, A.D.R. (2023). Sun protection and antibacterial activities of carotenoids from the soft coral Sinularia sp. symbiotic bacteria from Panjang Island, North Java Sea. Saudi Pharmaceutical Journal, 31(8): 101680. https://doi.org/10.1016/j.jsps.2023.06.013

[4] Pringgenies, D., Setyati, W.A., Ismail, A., Ariyanto, D. (2024). Hair cosmetic ingredients from the symbiont bacteria Xylocarpus sp. fruit are based on antimicrobial and antifungal bacteria. Journal of Applied Pharmaceutical Science, 14(5): 211-219. https://doi.org/10.7324/japs.2024.164624

[5] Kim, S. (2014) Marine cosmeceuticals. Journal of Cosmetic Dermatology, 13: 56-67. https://doi.org/10.1111/jocd.12057

[6] Ogé, L.K., Broussard, A., Marshall, M.D. (2019). Acne vulgaris: Diagnosis and treatment. American Family Physician, 100(8): 475-484.

[7] Pringgenies, D., Setyati, W.A., Feliatra, F., Ariyanto, D. (2023). The antibacterial and antifungal potential of marine natural ingredients from the symbiont bacteria of mangrove. Global Journal of Environmental Science and Management, 9(4): 819-832. https://doi.org/10.22035/gjesm.2023.04.11

[8] Kusmita, L., Mutmainah, N.F.N., Sabdono, A., Trianto, A., Radjasa, O.K., Pangestuti, R. (2021). Characteristic evaluation of various formulations of anti‐aging cream from carotenoid extract of bacterial symbiont Virgibacillus salarius strain 19.PP.Sc1.6. Cosmetics, 8(4): 120. https://doi.org/10.3390/cosmetics8040120

[9] Pavan, M.E., López, N.I., Pettinari, M.J. (2020). Melanin biosynthesis in bacteria, regulation and production perspectives. Applied Microbiology and Biotechnology, 104(4): 1357-1370. https://doi.org/10.1007/s00253-019-10245-y

[10] Dharma, B. (1988) Indonesian Shells II. PT. Sarana Graha, Indonesia.

[11] Pringgenies, D., Idris, R. (2019). The antioxidant activity of carotenoid pigments in the bacterial symbionts of seagrass Syringodium isoetifolium. Indonesian Journal of Natural Pigments, 1(1): 12-15. https://doi.org/10.33479/ijnp.2019.01.1.12

[12] Huang, X., Ge, X., Zhou, L., Wang, Y. (2022). Eugenol embedded zein and poly(lactic acid) film as active food packaging: Formation, characterization, and antimicrobial effects. Food Chemistry, 384: 132482. https://doi.org/10.1016/j.foodchem.2022.132482

[13] Yang, X., Wang, Z., Zhang, C., Wang, L., Pang, L., Zhang, D., Man, C., Jiang, Y. (2021). Assessment of the production of Bacillus cereus protease and its effect on the quality of ultra-high temperature-sterilized whole milk. Journal of Dairy Science, 104(6): 6577-6587. https://doi.org/10.3168/jds.2020-19818

[14] Stavrianidi, A. (2020). A classification of liquid chromatography mass spectrometry techniques for evaluation of chemical composition and quality control of traditional medicines. Journal of Chromatography A, 1609: 460501. https://doi.org/10.1016/j.chroma.2019.460501

[15] Kazimierczak, P., Golus, J., Kolmas, J., Wojcik, M., Kolodynska, D., Przekora, A. (2022). Noncytotoxic zinc-doped nanohydroxyapatite-based bone scaffolds with strong bactericidal, bacteriostatic, and antibiofilm activity. Biomaterials Advances, 139: 213011. https://doi.org/10.1016/j.bioadv.2022.213011

[16] Pringgenies, D., Santosa, G.W., Djunaedi, A., Susanto, A.B. (2021). Potential of bioactive compounds of holothuria atra- associated bacteria as a raw material in bioindustry. New Visions in Biological Science, 4: 66-75. https://doi.org/10.9734/bpi/nvbs/v4/4436f

[17] Zhukova. N.V., Eliseikina. M.G., Balakirev, E.S., Ayala, F.J. (2022). Multiple bacterial partners in symbiosis with the nudibranch mollusk Rostanga alisae. Scientific Reports, 12(1): 169. https://doi.org/10.1038/s41598-021-03973-7

[18] Huang, R., Crowther, T.W., Sui, Y., Sun, B., Liang, Y. (2021). High stability and metabolic capacity of bacterial community promote the rapid reduction of easily decomposing carbon in soil. Communications Biology, 4(1): 1376. https://doi.org/10.1038/s42003-021-02907-3

[19] Ahn, J.H., Seo, H., Park, W., Seok, J., Lee, J.A., Kim, W.J., Kim, G.B., Kim, K.J., Lee, S.Y. (2020). Enhanced succinic acid production by Mannheimia employing optimal malate dehydrogenase. Nature Communications, 11(1): 1970. https://doi.org/10.1038/s41467-020-15839-z

[20] Wang, T., Huang, Q., Burns, A.S., Moran, M.A., Whitman, W.B. (2022). Oxidative stress regulates a pivotal metabolic switch in dimethylsulfoniopropionate degradation by the marine bacterium Ruegeria pomeroyi. Microbiology Spectrum, 10(6): e03191-22. https://doi.org/10.1128/spectrum.03191-22

[21] Yu, Y., Wang, P., Cao, H.Y., Teng, Z.J., Zhu, Y., Wang, M., McMinn, A., Chen, Y., Xiang, H., Chen, X.L.,

Zhang, Y.Q. (2023). Novel D-glutamate catabolic pathway in marine Proteobacteria and halophilic archaea. ISME Journal, 17(4): 537-548. https://doi.org/10.1038/s41396-023-01364-6

[22] Qamar, A., Mysore, K.S., Senthil-Kumar, M. (2015). Role of proline and pyrroline-5-carboxylate metabolism in plant defense against invading pathogens. Frontiers in Plant Science, 6: 503. https://doi.org/10.3389/fpls.2015.00503

[23] Gupta, A., Patil, M., Qamar, A., Senthil-Kumar, M. (2020). ath-miR164c influences plant responses to the combined stress of drought and bacterial infection by regulating proline metabolism. Environmental and Experimental Botany, 172: 103998. https://doi.org/10.1016/j.envexpbot.2020.103998

[24] Jensen, J.V.K., Eberhardt, D., Wendisch, V.F. (2015). Modular pathway engineering of Corynebacterium glutamicum for production of the glutamate-derived compounds ornithine, proline, putrescine, citrulline, and arginine. Journal of Biotechnology, 214: 85-94. https://doi.org/10.1016/j.jbiotec.2015.09.017

[25] Ojovan, B., Catana, R., Neagu S., Cojoc, R., Lucaci, A.I., Marutescu, L., Florescu, L., Ruginescu, R., Enache, M., Moldoveanu, M. (2021). Metabolic potential of some functional groups of bacteria in aquatic urban systems. Fermentation, 7(4): 242. https://doi.org/10.3390/fermentation7040242

[26] Devi, S.P., Jha, D.K. (2020). Isolation of a lipolytic and proteolytic bacillus licheniformis from refinery oily sludge and optimization of culture. Microbiology & Biotechnology Letters, 48(4): 515-524. https://doi.org/10.48022/mbl.2004.04007

[27] Sahariah, B., Sarma, B.K. (2019). Relative orientation of the carbonyl groups determines the nature of orbital interactions in carbonyl-carbonyl short contacts. Chemical Science, 10(3): 909-917. https://doi.org/10.1039/c8sc04221g

[28] Gao, B., Jin, M., Li, L., Qu, W., Zeng, R. (2017). Genome sequencing reveals the complex polysaccharide-degrading ability of novel deep-sea bacterium Flammeovirga pacifica WPAGA1. Frontiers in Microbiology, 8: 600. https://doi.org/10.3389/fmicb.2017.00600

[29] Pardo-esté, C., Cortés, J., Castro-severyn, J., Pérez, V., Henriquez-aedo, K., Cuadros, F., Yañez, C., Cuadros-orellana, S., Dorador, C., Molina, V., Eissler, Y., Paquis, P., Jeffrey, W.H., Pozo, P., Pérez, P.A., Hengst, M.B. (2024). Secondary metabolites with antimicrobial activity produced by thermophilic bacteria from a high-altitude hydrothermal system. Frontiers in Microbiology, 15: 1477458. https://doi.org/10.3389/fmicb.2024.1477458

[30] Cai, X., Sun, H., Yan, B., Bai, H., Zhou, X., Shen, P., Jiang, C. (2023). Salt stress perception and metabolic regulation network analysis of a marine probiotic Meyerozyma guilliermondii GXDK6. Frontiers in Microbiology, 14: 1193352. https://doi.org/10.3389/fmicb.2023.1193352