https://doi.org/10.48048/tis.2024.7394

The potential Two Types of Green Macroalgae (*Caulerpa racemosa* and *Caulerpa lentillifera*) as a Natural Food Preservative from Jepara beach, Indonesia

Gunawan Widi Santosa[1], Ali Djunaedi[1], AB Susanto[1], Delianis Pringgenies[1*], Dafit Ariyanto[2] and Aris Kabul Pranoton[3]

[1]*Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Central Java 50275, Indonesia*
[2]*Research Center for Oceanography, National Research and Innovation Agency, Central Jakarta 14430, Indonesia*
[3]*Marine Engineering and Fisheries Polytechnic Karawang, Ministry of Marine and Fisheries, West Java 41315, Indonesia*

(*Corresponding author's e-mail: delianispringgenies@lecturer.undip.ac.id)

Received: 15 August 2023, Revised: 23 November 2023, Accepted: 30 November 2023, Published: 5 March 2024

Abstract

Green macroalgae, known locally as Latoh, is one of the green seaweeds consumed by the local community in Jepara and is beneficial for health. This study explores the potential of secondary metabolites from seaweed and its symbiotic bacteria as natural food preservatives and antibacterials. Seaweed samples were collected from the seagrass ecosystem of Panjang Island, Jepara, Indonesia. Subsequently, the samples were subjected to scanning electron microscopy analysis, proximate analysis, phytochemical analysis, thin layer chromatography, and high-performance liquid chromatography for amino acid analysis. A sample was subjected to a multistage extraction process using n-hexane, ethyl acetate, and methanol (1:5 w/v), each for 24 h. Symbiotic bacteria from seaweed were isolated, and enzymatic (proteolytic, amylolytic, and cellulolytic) and antibacterial testing against pathogenic bacteria *Staphylococcus aureus* and *Escherichia coli* was conducted using the disc diffusion method. The selected bacteria were subjected to molecular identification. The research showed that *Caulerpa lentillifera* had an ash content of 3.24 %, protein content of 0.57 %, and fat content of 0.337 %. Phytochemical analysis shows that the sample contains flavonoids, steroids, and alkaloids. HPLC analysis reveals that *Caulerpa lentillifera* has the highest content of aspartic acid (relative area: 11.90 %), glutamic acid (relative area: 13.43 %), and alanine (relative area: 9.03 %). *Caulerpa racemosa* sample shows the highest detector response for glutamic acid (relative area: 12.19%), aspartic acid (relative area: 11.10 %), and alanine (relative area: 9.63 %). The results indicate that 14 bacterial isolates were successfully isolated, with 6 isolates from *Caulerpa lentillifera* and 8 isolates from *Caulerpa racemosa,* all exhibiting enzymatic and antibacterial abilities. The research results concluded that the Latoh seaweed species *Caulerpa lentillifera* and *Caulerpa racemosa* and their symbiotic bacteria have the potential to be used as food preservatives.

Keywords: Food preservatives, HPLC, Phytochemical analysis, Proximate analysis, Secondary metabolite, Seaweed, Symbiotic bacteria

Introduction

Seaweed, biologically, is one of the groups of macroalgae that have chlorophyll. Many types of seaweed are found along the coast of Indonesia, and it is known to be very diverse throughout the Indonesian coast. However, not all of them are utilized by the Indonesian community. The widely used and collected seaweed for export are *Gelidium, Gelidiella, Hypnea, Euchema, and Fifaciforio*. Generally, these seaweeds are utilized as food [1], immune response of fish [2] and medicinal ingredients [3]. The commonly found types of seaweed are red, brown, and green. Green seaweed found on Jepara, Central Java, Indonesia's coast, is often consumed as a vegetable and is locally known as latoh. Latoh has great potential for development and is known to contain high levels of iodine minerals [4], amino acid [5] and antioxidant [6].

Seaweed from Latoh can now be branded as originating from Jepara and will be able to penetrate the international market, specifically Japan and Korea. Seaweed, particularly the Latoh variety, is essential because it contains polysaccharide compounds. Polysaccharides are essential because they provide several

important functions within the body. However, most of these carbohydratesconsist of gum compounds that are difficult to digest for humans [7]. On the other hand, seaweed is a flora that is required by the food [8], pharmaceutical [9,10], and cosmetic industries [11]. Since ancient times, coastal communities in Jepara and its surroundings have used seaweed as a vegetable. Seaweed is in high demand in the food industry, even in the Karimunjawa Islands and their surroundings. *Caulerpa sp.* of direct observations have found large-scale seaweed cultivation specifically for food [12,13].

Bacteria quickly spoil several types of food. Food spoilage is caused by the growth and development of bacterial spores in the food, characterized by changes in odour and taste, pH changes, and gas production [14,15]. This usually occurs in types of food with high water content. Synthetic preservatives are widely used to address this problem. Still, the Food and Drug Supervisory Agency (BPOM) does not recommend them because they can have side effects on consumers if consumed too frequently. Therefore, natural food preservative alternatives are needed by utilizing secondary metabolites in macroalgae. Some secondary metabolite compounds produced by macroalgae include alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, quinones, lipids, glycerol, rutin, quercetin, and kaempferol [16,17]. These compounds can inhibit the growth of bacteria that cause food spoilage. Natural preservatives and anti-bacterial ingredients from seaweed have been widely used in various products, especially in the cosmetic, food, and pharmaceutical industries. Seaweed is a very abundant natural resource rich in bioactive compounds with antimicrobial and antioxidant properties. Compounds such as alginic acid, fucoidan, and carrageenan in seaweed have been proven to inhibit the growth of microorganisms that cause product damage. The antioxidant content in seaweed also helps maintain product stability and prevent oxidation. Based on the previous description, the purpose of this research is to determine the potential of secondary metabolites from seaweed and its symbiotic bacteria as natural food preservatives and antibacterials**.**

Materials and methods

Research site and sample preparation

A total of 5 kg of seaweed samples were collected from the marine ecosystem on Panjang Island, Jepara, Indonesia. Sampling was not carried out in the summer, namely September 2022. Seaweed in the summer is usually not submerged in water, which affects its quality. The samples were then placed in a plastic zip lock bag containing seawater and taken to the laboratory for identification. A seaweed sample is washed with fresh water to remove salt and impurities and dried using air drying without direct sunlight. Next, the piece is blended into powder and extracted using a multi-level maceration method with solvents of n-hexane, ethyl acetate, and methanol (1:5 w/v), each for 24 h [18].

Content analysis

Phytochemical analysis

Scanning Electron Microscopy (SEM) analysis was performed using dried seaweed samples. The seaweed extract was tested using phytochemical and Thin Layer Chromatography (TLC) assays [18]. Subsequently, High-Performance Liquid Chromatography (HPLC) using analysis of Amino Acids.

Symbiotic bacterial isolation

Symbiotic bacteria were isolated from seaweed samples. Before isolation, the seaweed samples were washed thoroughly with fresh water so that there were no other microorganisms and therefore seaweed symbiont bacteria were obtained. Seaweed samples were crushed using a blender to obtain a seaweed cell suspension. Next, 0.10 mL of the sample was mixed with 0.90 mL of saline solution and the sample was incubated on agar medium. Bacterial colonies that grew from inoculation using Zobell 2216 E media were then purified using the streak method until obtaining pure settlements. The characteristics of the bacteria were determined by macroscopic observation based on the pure culture's size, colour, elevation, form, and margins [19].

Enzymatic quantitative test

Enzymatic testing was conducted on pure colonies of symbiotic bacteria from seaweed. The activities of amylase, protease, and cellulase enzymes were tested using Zobell 2216 E media enriched with the paper disc method. The amylase activity test was conducted using media enriched with 1 % starch. A 1 % iodine lugol solution was poured onto the media to detect amylase activity. The protease test was conducted using media enriched with 1 % skim milk. The cellulase activity test was conducted by adding 1% CMC

(Carboxymethylcellulose) to the media and pouring a congo red solution onto the press to indicate clear zones resulting from the produced enzymes [20].

Antibacterial quantitative test and bacterial identification

Antibacterial activity testing was conducted based on the disc diffusion method according to Pringgenies *et al.* [21] with some modifications. This antibacterial activity testing was performed to determine the ability of bacterial isolates to inhibit the growth of the pathogenic bacteria Staphylococcus aureus and Escherichia coli. The pathogenic bacteria were previously cultured on Nutrient Agar (NA) and then cultured in Nutrient Broth (NB) for 24 h. The pathogenic bacteria in the NB medium were then evenly spread onto Mueller Hinton Agar (MHA) using a sterile cotton swab. A paper disc was placed on the agar and the bacterial isolate that had been cultured in liquid form was inoculated onto the paper disc in a volume of 20 µL. The test medium was incubated and the formation of inhibition zones around the paper disc was observed and measured every 24 h for a total of 3 days using calipers, and documented.

DNA extraction, PCR and sequencing

The process of extracting DNA from symbiotic bacteria in seaweed is carried out by adding an extraction kit to a pure colony of bacteria until obtaining the supernatant of the solution. A total of 50µl of the extracted DNA swas used for PCR process. PCR analysis and electrophoresis are performed to get results in the form of DNA amplification. The amplified results are sent to PT. Genetika Science Indonesia, where the nucleotide base sequence will be identified [19].

Identification of symbiotic bacteria

The DNA seuences are then analyzed using MEGA 11 software to get a phylogenetic tree. The FASTA data of the nucleotide base sequences are compared with the data from the GenBank National Center for Biotechnology Information (NCBI) to identify the relationships between species [18].

Gas chromatography-mass spectrometry (GC-MS) symbiotic bacteria

Mass culture of pure colonies of symbiotic seaweed bacteria was conducted using Marine Broth media and extracted using the methanol maceration method. The concentrated extract was then analyzed for compound content using Gas Chromatography-Mass Spectrometry (GC-MS) technique. The analysis was performed using the GCMS-QP2010 SE: SHIMADZU instrument.

Results and discussion

The cultivation of latoh or *Caulerpa* sp is collected from the fish farmers currently harvesting seaweed in the waters of Jepara, located at latitude 6°36'46.8"S and longitude 110°33'32.9"E. The collected seaweed is very fresh and excellent for immediate research analysis as showed **Figure 1**.

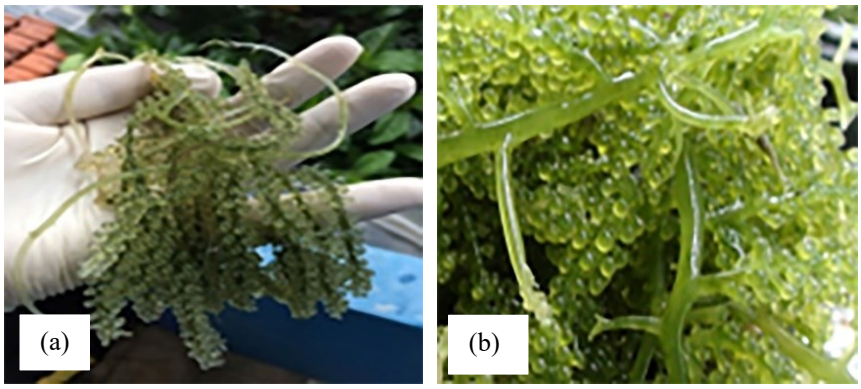
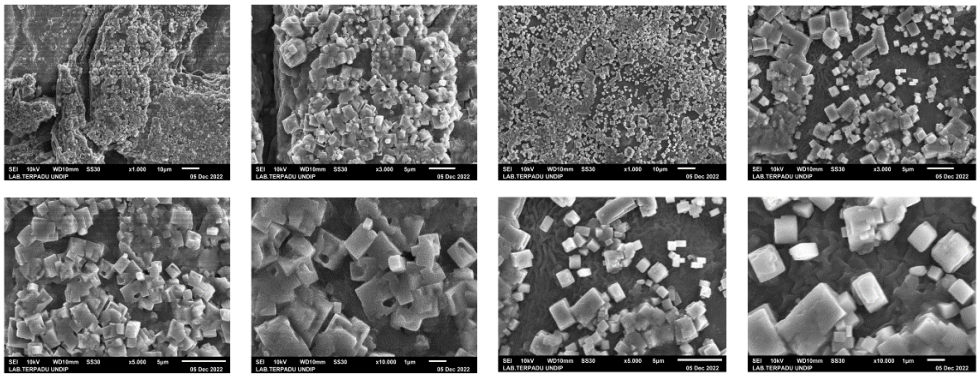


Figure 1 (a) *C. lentillifera* and (b) *C. racemose*.

Morphology

The identification results reveal that the seaweed found in Panjang Island, Jepara waters consists of 2 types: *Caulerpa racemosa* (Forsskål) J. Agardh, 1873) and *Caulerpa lentillifera* (J. Agardh, 1837). *C. racemosa* seaweed is characterized by its green, round-shaped thallus and ramuli, with a stem measuring approximately 15 - 22 cm. The species C. *lentillifera*, on the other hand, has bright green ramuli that

resembled grapes. Based on the image obtained from SEM, particles can be seen on the surface of seaweed ramuli. The SEM test results show that the sample is still fresh and has lots of salt particles even though it has been washed. This means that even if eaten as a side dish, the salty taste of the vegetables is still there and adds to the taste as shown in **Figure 2**.



(a) (b)

Figure 2 Scanning electron microscopy (SEM) of (a) *C. lentillifera* and (b) *C. racemose*.

Phytochemical analysis

Phytochemical analysis was conducted from the extract of *C. lentillifera*, where flavonoid and steroid compounds were successfully extracted in the ethyl acetate and methanol fractions. Additionally, alkaloid compounds were also detected in the ethyl acetate maceration sample. Tannin compounds were detected in the ethyl acetate maceration of *C. lentillifera*. The piece does not contain tannin. Based on the HPLC analysis of amino acids in the *C. lentillifera* sample, the highest detector response was observed for aspartic acid (relative area: 11.90 %), glutamic acid (close area: 13.43 %), and alanine (relative area: 9.03 %) as shown in **Table 1**. The *C. racemosa* sample had the highest detector response in the following order: glutamic acid (close location: 12.19 %), aspartic acid (relative area: 11.10 %), and alanine (close area: 9.63 %), as shown in **Table 2**.

Table 1 HPLC integration results of *C. lentillifera* amino acids.

No.	Peak name	Retention time (min)	Area (counts×min)	Height counts	Relative area (%)	Relative height (%)
1	Aspartic acid	2.55	144238.56	1147070.26	11.90	18.06
2	Glutamic acid	4.77	162811.97	884020.28	13.43	13.92
3	Serine	10.11	90762.33	354599.36	7.49	5.58
4	Histidine	11.47	8439.81	30680.95	0.70	0.48
5	Glycine	14.22	108955.44	363913.25	8.99	5.73
6	Threonine	15.13	85543.05	295809.92	7.06	4.66
7	Arginine	15.98	59787.81	212705.72	4.93	3.35
8	Alanine	18.83	109424.12	459782.75	9.03	7.24
9	Tyrosine	20.73	32620.64	196556.57	2.69	3.09
10	Methionine	24.60	14127.47	99172.67	1.17	1.56
11	Valine	24.95	103613.84	603472.60	8.55	9.50
12	Phenylalanine	25.82	48935.19	317338.09	4.04	5.00
13	Isoleucine	27.01	66573.42	420056.63	5.49	6.61
14	Leucine	27.52	84914.25	563030.04	7.01	8.86
15	Lvsine	30.02	9295.74	81630.71	0.77	1.29

A total of 14 *Caulerpa* sp. bacterial symbiont isolates were successfully purified, including 6 isolates of *C. lentillifera* (isolate code CL) and eight isolates of *C. racemosa* (isolate code CR). The dominant morphological character of the bacterial colony size observed was minor, with only CL21 and CR44 having a moderate extent. The colour of the various bacterial colonies was primarily white (9 isolates) and yellow (4 isolates), but one isolate CL21 had a whitish-yellow colour. Isolates CL22, CR31, and CR32 had the characteristics of small size, yellow colour, raised elevation, round form, and entire margin. Isolates CL31 and CR41 had the same features: Small size, white colour, flat elevation, round form, and entire margin.

Table 2 HPLC integration results of *C. racemosa* amino acid.

No.	Peak name	Retention time (min)	Area (counts×min)	Height counts	Relative area (%)	Relative height (%)
1	Aspartic acid	2.587	166599.28	1266376.37	11.10	16.50
2	Glutamic acid	4,830	183001.40	1002824.19	12.19	13.06
3	Serine	10,157	114233.07	444855.15	7.61	5.79
4	Histidine	11,533	13516.37	50617.06	0.90	0.66
5	Glycine	14,273	134256.46	450198.08	8.94	5.86
6	Threonine	15.17	104023.92	361886.95	6.93	4.71
7	Arginine	16.02	66900.82	237815.44	4.46	3.10
8	Alanine	18.86	144584.98	604415.91	9.63	7.87
9	Tyrosine	20.76	41835.03	253967.77	2.79	3.31
10	Methionine	24.62	16408.48	115454.86	1.09	1.50
11	Valine	24.97	137918.33	761580.89	9.19	9.92
12	Phenylalanine	25.85	56276.46	364229,52	3.75	4.74
13	Isoleucine	27.04	78680.36	492617.61	5.24	6.42
14	Leucine	27.55	96714.10	645109.17	6.44	8.40
15	Lvsine	30.06	16847.53	143245.56	1.12	1.87

Table 3 Characterization of seaweed symbiotic bacteria isolates.

No.	Isolate code	Morphology				
		Size	Colour	Flat/Raised elevation	Form	Margin
1	CL21	Moderate	White Yellowish	Flat	Round	Undulate
2	CL22	Small	Yellow	Raised	Round	Entire
3	CL31	Small	White	Flat	Round	Entire
4	CL32	Small	White	Flat	Punctiform	Entire
5	CL33	Small	White	Raised	Round	Entire
6	CL42	Small	White	Flat	Filamentous	Entire
7	CR11	Small	White	Flat	Punctiform	Entire
8	CR31	Small	Yellow	Raised	Round	Entire
9	CR32	Small	Yellow	Raised	Round	Entire
10	CR33	Small	White	Flat	Irregular	Entire
11	CR41	Small	White	Flat	Round	Entire
12	CR42	Small	White	Flat	Punctiform	Entire
13	CR43	Small	Yellow	Convex	Round	Entire
14	CR44	Moderate	White	Flat	Filamentous	Filamentous

Enzymatic assay of bacterial isolates from seaweed showed the highest proteolytic enzyme activity at each observation time was CL32 at 18 h (0.68±0.085 mm) and 24 h (0.975±0.148 mm), as well as CR31 at 30 h (0.975±0.148 mm). The isolate with the highest amylolytic enzyme ability was CL42 at 24 h (0.798±0.608 mm) and 48 h (1.165±0.106 mm) observation time. The most considerable cellulolytic enzyme assay result was found in isolate CL32 at both observation times, namely 24 h (0.815±0.0092 mm) and 48 h (1.515±0.064 mm). The results of the enzymatic assay are presented in **Table 4**.

Table 4 Enzymatic test of seaweed symbiotic bacteria isolates of inhibition zone (mm).

Code	Proteolytic			Amylolytic		Cellulolytic	
	18 h	24 h	30 h	24 h	48 h	24 h	48 h
CL21	0.02±0.028	0±0	0±0	0.713±0.587	1.01±0.127	0.2±0.283	0.335±0.064
CL22	0.64±0.212	0.725±0.064	0±0	0.186±0.283	0.05±0.07	0.25±0.354	0.46±0.113
CL31	0.49±0,58	0.535±0.658	0±0	0.345±0.488	0.716±0.419	0.4±0.556	0.415±0.12
CL32	0.68±0.085	0.975±0.148	0±0	0.56±0.028	0.35±0.467	0.815±0.0092	1.515±0.064
CL33	0.17±0.24	0.25±0.354	0±0	0±0	0.15±0.17	0.115±0.163	0±0
CL42	0±0	0±0	0±0	0.798±0.608	1.165±0.106	0.19±0.269	0.225±0.318
CR11	0.519±0.002	0.805±0.134	0.49±0.693	0.57±0.042	0.362±0.322	0.17±0.24	0±0
CR31	0.25±0.12	0.23±0.26	0.64±0.662	0.058±0.162	0±0	0.475±0.177	0.195±0.276
CR32	0.38±0.334	0.234±0.344	0.507±0.44	0,12±0	0.24±0.34	0.365±0.049	0.185±0.262
CR33	0±0	0±0	0±0	0±0	0±0	0.28±0.396	0.15±0.212
CR41	0±0	0±0	0±0	0.139±0.187	0.145±0.205	0.665±0.474	0.251±0.355
CR42	0±0	0±0	0±0	0.98±0.021	1.105±0.106	0.255±0.361	0.165±0.233
CR43	0.497±0.165	0±0	0.263±0.456	0.288±0.445	0.26±0.368	0.57±0.467	0.22±0.311
CR44	0±0	0±0	0±0	0.333±0.014	0.315±0.035	0.655±0.205	0.175±0.247

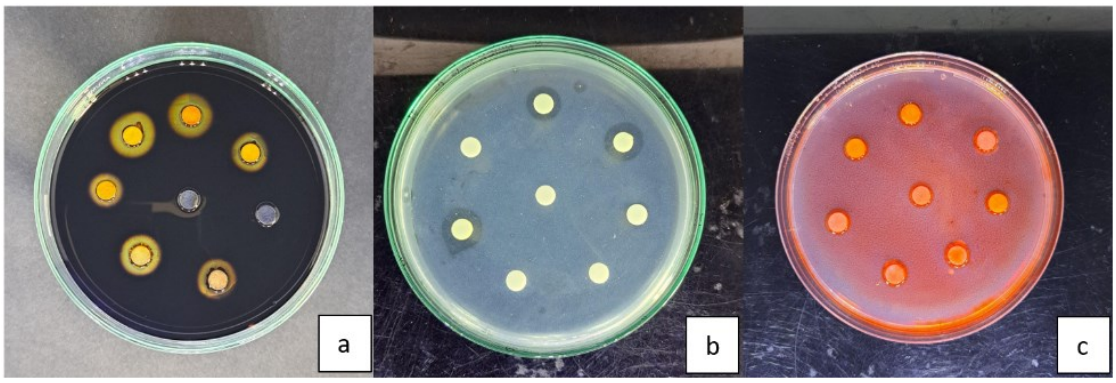


Figure 3 (a) Amylolytic, (b) proteolytic and (c) cellulolytic enzymatic tests.

The antibacterial test results on the bacterial isolates from seaweed symbionts against *S. aureus* and *E. coli* showed several clear zones. The largest clear zone against *S. aureus* at 24 h was shown by CL 22 (1.15±1.626 mm), at 48 h was CR32 (0.66±0 mm), and at 72 h was CR31 (0.53±0.75 mm). The observation at 24 h showed no activity in the clear zone against *E. coli*, but clear zones started to appear at 48 and 72 h. The largest antibacterial clear area was observed in CR 42 at 48 h (0.95±0.042 mm) and CL21 at 72 h (0.15±0.212 mm). Isolates CL31 and CL32 could not form antibacterial activity against *S. aureus* and *E. coli* in the antibacterial test, as seen in **Table 5**.

Table 5 Antibacterial test of seaweed symbiotic bacteria isolates.

No.	Isolate code	Inhibition zone (mm)					
		Staphylococcus aureus			***Escherichia coli***		
		24 h	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
1	RL42	0±0	0.03±0.042	0±0	0±0	0±0	0±0
2	CL21	0.25±0.354	0.05±0.071	0±0	0±0	0±0	0.15±0.212
3	CL22	1.15±1.626	0±0	0±0	0±0	0±0	0±0
4	CL31	0±0	0±0	0±0	0±0	0±0	0±0
5	CL32	0±0	0±0	0±0	0±0	0±0	0±0
6	CL33	0.4±0.566	0.37±0.014	0.19±0.269	0±0	0±0	0±0
7	CR11	0.2±0.283	0.43±0.608	0.43±0.608	0±0	0±0	0±0
8	CR31	0.6±0.849	0.61±0.636	0.53±0.75	0±0	0.59±0.156	0.11±0.156
9	CR32	0.25±0.354	0.66±0	0.33±0.467	0±0	0.03±0.042	0.05±0.071
10	CR33	0±0	0±0	0±0	0±0	0.01±0.014	0.09±0.127
11	CR41	0±0	0±0	0±0	0±0	0.25±0.354	0±0
12	CR42	0±0	0±0	0±0	0±0	0.95±0.042	0±0
13	CR43	0±0	0.13±0.184	0.13±0.184	0±0	0.45±0.495	0±0
14	CR44	0±0	0.23±0.325	0±0	0±0	0.54±0.622	0±0

Identification of symbiotic bacteria

The sequencing results of 6 selected bacterial isolates, 3 isolates from code CL and 3 isolates from code CR, are presented in **Table 6**. There were 6 bacteria identified based on the results of the best antibacterial test and enzymatic test. The symbiotic bacteria identified in seaweed *are Pseudoalteromonas arabiensis* (CL21, 99.79 % and CR33, 100 %), *Pseudoalteromonas piscicida* (CL22, 99.78 % and CR31, 99.93 %), *Pseudoalteromonas shioyasakiensis* (CL33, 99.23 %), and *Vibrio alginolyticus* (CR44, 99.90 %), as stated in **Table 6**.

Table 6 Identification Results of Seaweed Symbiotic Bacteria.

Code	Description	Scientific name	Query cover	Per. Ident	Accession
CL21	*Pseudoalteromonas arabiensis* strain k53 16S ribosomal RNA, partial sequence	*Pseudoalteromonas arabiensis* strain k53	100 %	99.79 %	OR253445
CL22	*Pseudoalteromonas piscicida* strain NBRC 103038 16S ribosomal RNA, partial sequence	*Pseudoalteromonas piscicida* strain NBRC 103038	100 %	99.78 %	OR253446
CL33	*Pseudoalteromonas shioyasakiensis* strain SE3 16S ribosomal RNA, partial sequence	*Pseudoalteromonas shioyasakiensis* strain SE3	100 %	99.23 %	OR253447
CR31	*Pseudoalteromonas piscicida* strain NBRC 103038 16S ribosomal RNA, partial sequence	*Pseudoalteromonas piscicida* strain NBRC	100 %	99.93 %	OR253448
CR33	*Pseudoalteromonas arabiensis* strain k53 16S ribosomal RNA, partial sequence	*Pseudoalteromonas arabiensis* strain k53	100 %	100.00 %	OR253449
CR44	*Vibrio alginolyticus* strain NBRC 15630 16S ribosomal RNA, partial sequence	*Vibrio alginolyticus* strain NBRC 15630	100 %	99.90 %	OR253450

The types of seaweed symbiont bacteria found are known to be found in sediments, such *as Pseudomonas aeruginosa* bacteria found in mangrove sediments [22]. In unextracted seaweed of *Caulerpa,* the composition mainly consists of water, carbohydrates, proteins, fiber, and minerals. Water is the main component in this seaweed. However, in extracted Caulerpa, the composition can be more concentrated. Extraction is the process of obtaining specific substances from a material. For Caulerpa, extraction is usually done to obtain desired compounds.

Phytochemical analysis was conducted on the extract of *C. lentillifera*, which contains flavonoid and steroid compounds. Flavonoids, in particular, have been extensively utilized as antifungal and antibacterial agents [23], while steroid compounds are known to possess antibacterial activity [24]. The seaweed found at the sampling site is *C. lentillifera* and *C. racemosa*. As the results of research that has been carried out show that seaweed *Turbinaria decurrens*, *Globigerina rugosa* and *Halimeda. opuntia* contain flavonoids and alkaloids which have the potential to inhibit the growth of pathogenic bacteria in food [25].

The HPLC analysis extract of amino acids from *C. lentillifera* showed that the highest content was glutamic acid (13.43 %), aspartic acid (11.90 %), and alanine (9.03 %), which are non-essential amino acids [26]. Glutamic acid, aspartic acid, and alanine have been found in several studies to have antibacterial effects, particularly bactericidal antibacterial activities. These compounds act as antibacterials against gram-positive and gram-negative pathogenic bacteria [27-29].

The research found that 14 bacterial isolates were successfully isolated from *C. lentillifera* and *C. racemosa* seaweed and but 6 bacteria had the best antibacterials and ezymatic test. The enzymatic tests conducted on the bacterial isolates were proteolytic, amylolytic, and cellulolytic tests. The symbiotic isolates of seaweed showed that the most considerable proteolystic enzyme activity was found in isolate CL32 at 18 and 24 h of observation, while at 30 h of observation, the most significant action was found in CR31 (0.975±0.148 mm). The antibacterial tests on the bacterial isolates were conducted against the pathogenic bacteria *S. aureus* and *E. coli*. The largest clear zone against *S. aureus* was found in CL22 (1.15±1.626 mm) at 24 h, CR32 (0.66±0 mm) at 48 h, and CR31 (0.53±0.75 mm) at 72 h. The antibacterial activity at 24 h of observation against *E. coli* did not show positive results in any isolate. At 48 h of observation, the most considerable antibacterial sactivity was found in isolate CR42 (0.95±0).

The results of identifying symbiotic bacteria in seaweed showed that isolates CL21 and CR33 are species of *Pseudoalteromonas arabiensis.* This species is a type of gram-negative bacteria that is aerobic and commonly found in marine sediments. These bacteria produce polysaccharides and can convert nitrate into nitrite but not nitrogen [30]. The bacterial species *Pseudoalteromonas piscicida* was found in isolates CL22 and CR31. This species is a gram-negative bacterium that can be found in seawater. It is one of the pigmented bacteria with yellow colonies [31]. *P. piscicida* has been reported to have potential as a shrimp probiotic in aquaculture. This is due to its ability to enhance shrimp's immune response, growth, and resistance to the pathogen *Vibrio harveyi* [32]. This bacterium has also been reported to have the potential for hydrolysis of *Ulva lactuca* polysaccharides [33].

Isolate CL33 has been identified as *Pseudoalteromonas shioyasakiensis*. This bacterium, a gram-negative type capable of producing exopolysaccharides, is reported to be found in marine waters and sediments [30]. Out of 7 isolates, 6 isolates found in symbiotic bacteria of seaweed are Pseudomonas genus bacteria. *Pseudomonas sp.* bacteria are also frequently found in mangrove sediments, which have antibacterial properties [22].

The identification result of the CR44 isolate is *V. alginolyticus* species. *V. alginolyticus* is a halophilic gram-negative bacterium with a curved (comma) shape with a length of about 1.4 - 5.0 µm and a width of 0.3 - 1.3 µm. *V. alginolyticus* is motile and has flagella with a protective sheath [34]. This species is a natural host of estuarine and marine waters and is widely reported to be a pathogen for various diseases [35], such as wound infections, virulence, gastroenteritis, and other diseases. However, recent information explains that the bacterium *V. alginolyticus* has essential benefits in medical research, enzyme purification, fuel production, bioremediation, and environmental research. Further use and understanding of this bacterium can help develop better technology and applications in various industries and fields of science, as it has been found that the symbiotic bacterium *V. alginolyticus* has antibacterial activity against *S. aureus* and *E. coli* bacteria.

Many enzymes isolated from marine bacteria and macroalgae can be used to clean reverse osmosis membranes and detergents. The symbiotic bacteria *V. alginolyticus* produces collagenase and alkaline-resistant serine exoprotease, which have significant commercial applications in the chemical industry. Japanese researchers use advanced biotechnology processes to develop methods for inducing marine algae to produce larger quantities of superoxide dismutase, with applications in the biomedical industry, functional foods, and cosmetics [36]. Therefore, this type of bacteria can be beneficial as a food preservative. Symbiotic bacteria found in the seaweed species *C. lentillifera* and *C. racemosa* have the

potential as antibacterial agents. Due to their amino acid content, the seaweed extracts of *C. lentillifera* and *C. racemosa* can act as antibacterials. Therefore, the green macroalgae species *Caulerpa lentillifera* and *Caulerpa racemosa* have the potential as food preservatives.

Conclusions

The research findings concluded that the seaweed species *C. lentillifera* and *C.racemosa* contain flavonoid and steroid compounds. Seaweed extract can potentially be used as a food preservative due to its high content of essential amino acids. This means that seaweed types *C. lentillifera* and *C. racemosa* apart from being consumed as vegetables, are also useful for slowing food spoilage. This vegetable has a savory taste because there is salt content attached to the vegetable. There are 6 isolates of symbiotic bacteria found in the seaweed, 5 of which belong to the *Pseudomonas sp. P. arabiensis* strain k53 (99.79 %), *P. piscicida* strain NBRC 103038 (99.78 %), *P. shioyasakiensis* strain SE3 (99.23 %), *P. piscicida* strain NBRC (99.93 %), *Pseudoalteromonas arabiensis* strain k53 (100.00 %), and *V. alginolyticus* strain NBRC 15630 (99.90-%). All the bacteria species found have enzymatic activity and potential as antibacterial agents. Therefore, the research findings suggest that the seaweed species *C. lentillifera* and *C. racemosa*, along with their symbiotic bacteria, have the potential to be used as food preservatives.

Acknowledgements

We would like to thank for the Faculty Fisheries and Marine Science, Universitas Diponegoro, Indonesia for funding under No: 32/UN7. F10/PP/III/2023 and Dean No: 70/UN7.10/HK/III/2023.

References

[1] R Peñalver, JM Lorenzo, G Ros, R Amarowicz, M Pateiro and G Nieto. Seaweeds as a functional ingredient for a healthy diet. *Mar. Drugs* 2020; **18**, 301.
[2] AB Susanto, GW Santosa, A Djunaedi, D Pringgenies and D Ariyanto. The impact of mixed feed of seaweed *Gracilaria* sp. and transfer factor formula on the immune response of Nile tilapia (*Oreochromis niloticus*). *AACL Bioflux.* 2023; **16**, 242-51.
[3] Y Kumar, A Tarafdar and PC Badgujar. Seaweed as a source of natural antioxidants: Therapeutic activity and food applications. *J. Food Qual.* 2021; **2021**, 5753391.
[4] K Kasmiati, S Syahrul, B Badraeni and MH Rahmi. Proximate and mineral compositions of the green seaweeds *Caulerpa lentilifera* and *Caulerpa racemosa* from South Sulawesi Coast, Indonesia. *IOP Conf. Ser. Earth Environ. Sci.* 2022; **1119**, 012049.
[5] C Dawczynski, R Schubert and G Jahreis. Amino acids, fatty acids, and dietary fibre in edible seaweed products. *Food Chem.* 2007; **103**, 891-9.
[6] M Kumar, V Gupta, P Kumari, CRK Reddy and B Jha. Assessment of nutrient composition and antioxidant potential of Caulerpaceae seaweeds. *J. Food Compos. Anal.* 2011; **24**, 270-8.
[7] JN BeMiller. *Gums and related polysaccharides*. *In*: BO Fraser-Reid, K Tatsuta and J Thiem (Eds.). Glycoscience. Springer, Heidelberg, Germany.
[8] LE Rioux, L Beaulieu and SL Turgeon. Seaweeds: A traditional ingredients for new gastronomic sensation. *Food Hydrocoll.* 2017; **68**, 255-65.
[9] J Liu, S Luthuli, Q Wu, M Wu, J Il Choi and H Tong. Pharmaceutical and nutraceutical potential applications of sargassum fulvellum. *Biomed Res. Int.* 2020; **2020**, 13-5.
[10] D Pringgenies, EI 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.
[11] T Morais and D Pacheco. Seaweeds compounds: An ecosustainable source of cosmetic ingredient? *Cosmetics* 2021; **8**, 8.
[12] J Forster and R Radulovich. *Seaweed and food security*. *In*: BK Tiwari and DJ Troy (Eds.). Seaweed sustain. Academic Press, Massachusetts, 2015, p. 289-313.
[13] F Sultana and A Wahab. Seaweed farming for food and nutritional security, climate change mitigation and adaptation, and women empowerment: A review. *Aquac. Fish.* 2023; **8**, 463-80.
[14] S André, T Vallaeys and S Planchon. Spore-forming bacteria responsible for food spoilage. *Res. Microbiol.* 2017; **168**, 379-87.
[15] O Alegbeleye, OA Odeyemi, M Strateva and D Stratev. Microbial spoilage of vegetables, fruits and cereals. *Appl. Food Res.* 2022; **2**, 100122.
[16] MT Cabrita, C Vale and AP Rauter. Halogenated compounds from marine algae. *Mar. Drugs* 2010;

8, 2301-17.
[17] SSA llah Al-Saif, N Abdel-Raouf, HA El-Wazanani and IA Aref. Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. *Saudi J. Biol. Sci.* 2013; **21**, 57-64.
[18] AC Anggelina, D Pringgenies and WA Setyati. Presence of biosynthetic gene clusters (NRPS/PKS) in actinomycetes of mangrove sediment in Semarang and Karimunjawa, Indonesia. *Environ. Nat. Resour. J.* 2021; **19**, 391-401.
[19] WA Setyati, D Pringgenies, N Soenardjo and R Pramesti. Enzyme-producing symbiotic bacteria in gastropods and bivalves molluscs: Candidates for bioindustry materials. *Biodiversitas* 2023; **24**, 20-5.
[20] WA Setyati and Subagiyo. Isolation and selection of extracellular enzyme producing bacteria originating from mangrove sediment. *LMU Kelaut. Indones. J. Mar. Sci.* 2012; **17**, 164-9.
[21] D Pringgenies, AS Wilis, F Feliatra and D Ariyanto. The antibacterial and antifungal potential of marine natural ingredients from the symbiont bacteria of mangrove. *Glob. J. Environ. Sci. Manag.* 2023; **9**, 819-32.
[22] 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.
[23] M Azeem, M Hanif, K Mahmood, N Ameer, FRS Chughtai and U Abid. An insight into anticancer, antioxidant, antimicrobial, antidiabetic and anti-inflammatory effects of quercetin: A review. *Polym. Bull.* 2023; **80**, 241-62.
[24] A Vollaro, A Esposito, E Antonaki, VD Iula, D D'Alonzo, A Guaragna and ED Gregorio. Steroid derivatives as potential antimicrobial agents against staphylococcus aureus planktonic cells. *Microorganisms* 2020; **8**, 468.
[25] NS Chandel. Amino acid metabolism. *Cold Spring Harb. Perspect. Biol.* 2021; **13**, a040584.
[26] M Can, S Demirci, AK Sunol and N Sahiner. An amino acid, L-Glutamic acid-based metal-organic frameworks and their antibacterial, blood compatibility, biocompatibility, and sensor properties. *Microporous Mesoporous Mater.* 2020; **309**, 110533.
[27] A Pandey, A Devkota, Z Yadegari, K Dumenyo and A Taheri. Antibacterial properties of citric acid/β-alanine carbon dots against gram-negative bacteria. *Nanomaterials* 2021; **11**, 2012.
[28] J Shen, Z Zhou, D Chen, Y Wang, Y He, D Wang and J Qin. Poly(aspartic acid) based self-healing hydrogels with antibacterial and light-emitting properties for wound repair. *Colloids Surfaces B Biointerfaces* 2021; **200**, 111568.
[29] H Matsuyama, H Minami, H Kasahara, Y Kato, M Murayama and I Yumoto. *Pseudoalteromonas arabiensis* sp. nov., a marine polysaccharide-producing bacterium. *Int. J. Syst. Evol. Microbiol.* 2013; **63**, 1805-9.
[30] GP Richards, MA Watson, DS Needleman, J Uknalis, EF Boyd and P Fay. Mechanisms for *Pseudoalteromonas piscicida*-induced killing of vibrios and other bacterial pathogens. *Appl. Environ. Microbiol.* 2017; **83**, e00175-17.
[31] YI Nababan, M Yuhana, T Penataseputro, H Nasrullah, A Alimuddin and W Widanarni. Dietary supplementation of *Pseudoalteromonas piscicida* 1UB and fructooligosaccharide enhance growth performance and protect the whiteleg shrimp (*Litopenaeus vannamei*) against WSSV and *Vibrio harveyi* coinfection. *Fish Shellfish Immunol.* 2022; **131**, 746-56.
[32] NAE Azm, D Fleita, D Rifaat, EZ Mpingirika, A Amleh and MMH El-Sayed. Production of bioactive compounds from the sulfated polysaccharides extracts of *Ulva lactuca*: Post-extraction enzymatic hydrolysis followed by ion-exchange chromatographic fractionation. *Molecules* 2019; **24**, 2132.
[33] 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.
[34] U Yanuhar, H Nurcahyo, L Widiyanti, NS Junirahma, NR Caesar and S Sukoso. *In vivo* test of *Vibrio alginolyticus* and *Vibrio harveyi* infection in the humpback grouper (*Cromileptes altivelis*) from East Java Indonesia. *Vet. World* 2022; **15**, 1269-82.
[35] JPS Cabral. Water microbiology. Bacterial pathogens and water. *Int. J. Environ. Res. Publ.* 2010; **7**, 3657-703.
[36] A Rotter, M Barbier, F Bertoni, AM Bones, ML Cancela, J Carlsson, MF Carvalho, M Cegłowska, J Chirivella-Martorell, MC Dalay, M Cueto, T Dailianis, I Deniz, AR Díaz-Marrero, D Drakulovic, A Dubnika, C Edwards, H Einarsson, A Erdoğan, ..., M Vasquez. The essentials of marine biotechnology. *Front. Mar. Sci.* 2021; **8**, 1-53.