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Biodiversity of Symbiotic Microorganisms of *Caulerpa racemosa* from Lemukutan Island, Indonesia and Its Antibacterial Activity

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ABSTRACT
Symbiotic microorganisms usually have the same secondary metabolite activity as their host. The aims of this study were determine the biodiversity of symbiotic microorganisms from *Caulerpa racemosa* grown in Lemukutan Island, Indonesia and evaluate their antibacterial activity. Thirty eight bacterial and eighteen fungal were isolated. All isolates showed antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. All fungal isolates were identified from spore morphology using a microscope and, the best bacterial isolates with highest antibacterial activity (IB21 and IB47) were identified using biochemical tests, such as gram staining, citrate test, MR-VP test, carbohydrate fermentation test, fermentative oxidation test, oxidation test, catalase test, indole test, motility test, urease test and hydrogen production test (H2S). The genera of isolated fungi were identified as genus *Trichocladium, Aspergillus, Chaetomium, Coprinus, Cladorrhinum, Hymeochaete, Rhizopus, Tremella, Zygorhynchus, Mucor, and Bjerkandera.* Meanwhile the best bacteria IB21 and IB47 were putatively identified as member of genus *Corynebacterium* and *Neissiri*a, respectively.

Keywords: *Antibacterial Activity, Biodiversity, Caulerpa racemosa, Microorganism.*

1. INTRODUCTION

Indonesian marine waters have a high biodiversity of marine organisms, such as macroalgae. It was found 555 species out of 8,000 species of total world macroalgae biodiversity [1]. The diversity and community of macroalgae influenced by oceanographic, topographic, and biological factors [2]. Macroalgae are classified into three groups, namely brown (Phaeophyta), red (Rhodophyta) and green macroalgae (Chlorophyta). Macroalgae have a crucial role in primary productivity, absorbing pollutants, producing organic matter and oxygen for another aquatic biota. In addition, the ecological role of macroalgae is habitat for feeding, spawning, and nursery grounds [3] for other organisms.

Macroalgae is one of the marine resources in bioactive compounds. Macroalgae from the waters of West Kalimantan, namely *Eucheuma spinosum* from Lemukutan Island and *Padina pavonica* Hauck from Kabung Island have antioxidant activity [4],[5]. Antibacterial and antioxidant properties of *Caulerpa racemosa* and *C. lentillifera* have also been reported [6]. These bioactive compounds are secondary metabolites produced by macroalgae as a form of self-defense from unfavorable environmental conditions and pathogenic microbes. Bioactive compounds can also obtained from symbiotic microorganisms. Proksch [7] have reported that the bioactive compounds of symbiotic microorganisms are identical to those of the host. Therefore, symbiotic microorganisms have the potential as a source of bioactive compounds. Symbiont bacteria are more effective to use than crude extract of macroalgae because they are easy to culture in the laboratory, thus they can avoid excessive use of natural materials [8].

The bioactivity of bacterial and fungal symbionts has been widely reported. Antibacterial of CR2 bacterial isolates associated with C. racemosa was active against *Pseudomonas aeruginosa* and HM isolates associated with *Halimeda macroloba* were active against *Escherichia coli* and *P. aeruginosa* [9]. Bacteria associated with green macroalgae from Singkawang waters obtained 3 isolates of bacteria associated with *C. racemosa* and 4 isolates of bacteria from *Caulerpa taxifolia* had antibacterial activity [10]. Fungi symbionts

(FSUr-1, FSUr-2, FSUr-3) from *Ulva reticulata* from Takalar, South Sulawesi also showed antibacterial and antifungal activity [11] *Aspergillus nomius* associated with *Bornetella* sp. showed antibacterial against *E. coli* and *Staphylococcus aureus* [12]. The bioactivity of this symbiotic microorganisms indicates that the microorganism has great potential as an antibacterial. Therefore, the aimed of this research are determine the biodiversity of the symbiotic microorganisms of *C. racemosa* and its antibacterial activity.

2. MATERIALS

Materials used in this research were NB (Nutrient Broth), NA (nutrient agar), seawater, PDA (Potato Dextrose Agar), and Zobell 2216E, *C. racemosa*, bacteria test (*S. aureus* and *E. coli*), alcohol, aquades, set of biochemical test materials and phytochemical reagent kits, chloramphenicol (50 mg/L), and 1% sodium hypochlorite.

3. METHODS

3.1. *Sampling*

Samples of macroalgae *C. racemosa* was collected from Lemukutan Island, Indonesia, in 6 and 7 December 2020. The sample was taken at a depth of 0,9 - 1,3 meters with coordinate N 00˚ 46'48.46" E 108˚ 42'23.91" (Figure 1). The sample was put into a sterile plastic bag containing seawater and then stored in a cool box containing ice cubes for further analysis in the laboratory.

3.2. *Isolation of Microorganisms*

Isolation of fungi and bacteria was conducted using two methods, namely serial dilution, and direct method. The sample washed with flowing water and then the surface was sterilized by immersing the sample in 1% sodium hypochlorite solution for 5 minutes, 70% ethanol for 1 minute and in the end washed with sterile distilled water [20]. Sample crushed using a mortar and put into an erlenmeyer and added 100 mL sterile seawater (stock solution). The sample suspension from the stock solution was taken 1 mL and then put into 9 mL of sterile seawater to produce a 10^{-1} dilution, the same way was done to produce a 10^{-2} to 10^{-5} dilution [16]. The isolate with a dilution of 10^{-3} to 10^{-5} were inoculated with 1 mL in PDA (Potato Dextrose Agar) dissolved in distilled water and PDA dissolved in seawater, using pour plate methods [14], [18]. Chloramphenicol was added for inhibiting bacterial growth. Then, sample were incubated for 5-7 days at room temperature. For bacterial isolation, the isolate with the same dilution of 10^{-3} to 10^{-5} were inoculated with 1 mL in NA and Zobell 2116E by pour plate methods. After that, sample were incubated for 5-7 days at room temperature.

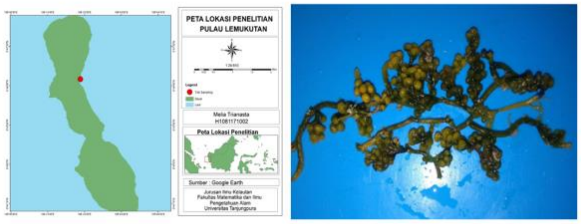


Figure 1 Sampling location (A), and *C. racemosa* (B).

Fungi isolation using direct method. The sample washed with flowing water and then the surface was sterilized by immersing the sample in a 1% sodium hypochlorite solution for 5 minutes, 70% ethanol for 1 minute and in the end washed with sterile distilled water [20]. Samples were inoculated directly on PDA surface agar. Samples were incubated for 5-7 days at room temperature. For bacterial isolation, samples inoculated on the NA and Zobell 2216E. Samples were incubated for 24 hours at room temperature.

3.3. *Screening Antibacterial Activity Test*

Antibacterial activity test of fungal isolates was carried out using the agar diffusion methods. Colonies of endophytic fungal isolates of green macroalgae C. racemosa were grown for 7 days then cut (6 mm in diameter) and placed on NA which had been spreading with bacterial test and incubated at 37ºC for 2 days. Antibacterial activity showed by formation of inhibition zone [19].

Antibacterial activity test of bacterial isolates was carried out by cross streak method. Colonies of bacteria were grown perpendicular to bacteria test and incubated at 37ºC for 1x24 hours. Antibacterial activity indicated by the formation of a clear zone [25].

3.4. Characterization of the Isolate Microorganism

Identification of endophytic fungal isolates were carried out based on identification book Pictorial Atlas of Soil and Seed Fungi Morphologies of Cultured Fungi and Key to Species, [22] and referring to the journal [21], [23], and [24]. Thus, macroscopic (colony color, and colony shape) and microscopic observations were conducted under a light microscope with a magnification of 100x [20].

Identification of bacterial isolates were using biochemical tests, namely gram staining, citrate test, MR-VP test, carbohydrate fermentation test (D(+)-glucose, sucrose, lactose, maltose, and D(+)-mannitol), fermentative oxidation test, oxidase test, catalase test, indole test, motility test, urease test and hydrogen sulphide test (H_2S).

3.5. Fermentation of Bacterial Suspension

Fermentation test used isolates using two bacteria with the highest antibacterial activity such as IB21 and IB47. The suspension of bacteria was grown in NB media, then agitated at 170 rpm for 24 hours. The suspension was centrifuged at 3,000 rpm for 30 minutes to separate the supernatant. The supernatant was used for phytochemical tests [35]

3.6. Phytochemical Activity Test of Bacterial Suspension

Phytochemical tests were conducted following to method by Masriani [37] to identify flavonoids, alkaloids, steroid/sterpenoids, sapponins, and phenolics compounds.

4. RESULT AND DISCCUSSION

4.1. Sampling and Isolation of Microorganisms

Samples of *C. racemosa* were taken under temperature of 29.53ºC, salinity of 32.37 ppt, pH of 8.046 and DO of 4.63 mg/L. A total of 38 of bacteria and 18 of fungi were isolated (Figure 2). Furthermore, the isolates were tested for antibacterial activity.

4.2. Screening of Antibacterial Activity

Screening of antibacterial activity of symbiotic microorganisms were conducted (Table 1). Two bacterial isolates (IB21 and IB47) showed highest antibacterial activity based on the formation of highest clear zone diameter. This is accordance with the research of Yap et al [6] *C. racemosa* had antibacterial activity against pathogenic bacteria (E.coli and S.aureus). Similar research carried out by Rahaweman et al. [14] 13 fungal isolates from macroalgae *Caulerpa* spp., *Halimeda* spp., and *Sargassum* spp. Kepulauan Seribu, Indonesia has antibacterial activity against pathogenic bacteria *S. aureus* and *E. coli*. Similar research was also conducted too by Ismail et al. [13]. A total of 26 bacterial isolates from the macroalga *Padina pavonica* had antibacterial activity against 12 pathogenic bacteria such as *S. aureus, E. coli, A. salmonicida, A. hydrophila, E. xiangfangensis, E. faecium, Micrococcus* sp., *S. typhimurium, Streptococcus* sp., *V. alginoliticus, V. proteolyticus*, and *V. vulnificus*. Another 19 isolates bacterial and fungi can inhibit only one of gram positive or negative pathogenic bacterial (Table 1). This could be due to differences in composition and structure of peptidoglycan in pathogen bacterial cell wall (*E. coli* and *S. aureus*) which can affect antibacterial activity [15].

4.3.Characterization of Microorganism

The biochemical test of IB21 (Table 2) showed positive result in metabolite products, namely: methyl red, O/F and simon citrate. IB21 was motile and gram-positive. Positive result in enzyme characteristic test, namely: oxidase and catalase, and negative of lactose test in carbohydrate fermentation. IB21 was bacilli. The results of the identification of bacteria based on Bergey's Manual of Determinative Bacteriology (1994) isolates IB21 suspected of the genera *Corynebacterium*. The biochemical test of IB47 (Table 2) show positive result in metabolite products, namely: indole, methyl red and O/F. IB47 had positive result in enzyme characteristic test, namely: oxidase and catalase, and positive result on sucrose and D(+)-glucose. IB47 was coccus, motile and gram-negative. The results of the identification, IB47 suspected of the genera *Neissiria*. *Corynebacterium* and *Neissiria* were obtained from marine environments such as algae [33], [38].

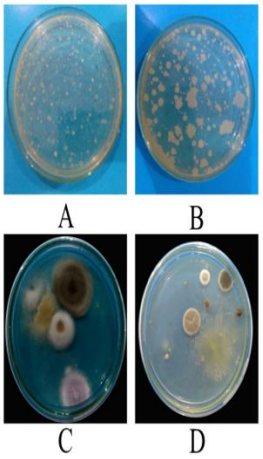


Figure 2. Symbiotic microorganisms (A,B) bacteria (C,D) fungi.

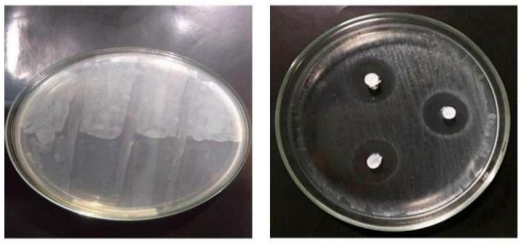
Endophytic fungal of *C. racemosa* from Lemukutan Island waters were identified macroscopically and microscopically. Macroscopic identification was carried out by looking at the color differences of fungi isolated colonies and light microscope with magnification of 100X (Table 3). The hyphae characteristics obtained from microscopic observations were matched with standard micrographs from identification books to determine the genus [22]. Based on the identification results, the genera of fungal isolates are *Trichocladium, Aspergillus, Chaetomium, Coprinus, Cladorrhinum, Hymeochaete, Rhizopus, Tremella, Zygorhynchus, Mucor, and Bjerkandera*. The most dominant genus of endophytic fungal isolates obtained was the genera *Aspergillus*. (Table 3).

Aspergillus commonly found in marine, such as macroalgae from different aquatic origins [26], [27], [28]. It was strengthened by the research of [29], [30], [31], that endophytic fungal of *C. racemosa* and sponges from India identified as the genera *Aspergillus, Penicillium, Cladosporium, Monascus*, and *Schizophyllum*. Other endophytic fungal genera and species were also identified by Ahamed and Murugan [28]; Handayani et al [32] from macroalgae namely *Chaetomium* and *Trichoderma harzianum*

Table 1. Screening of Antibacterial Activity

No.	Isolates	Medium	Antibacterial Activity	
			E. coli	*S. aureus*
1	IB02	NA	+	±
2	IB04	NA	+	±
3	IB06	NA	+	-
4	IB08	NA	±	-
5	IB09	NA	+	-
6	IB10	NA	±	±
7	IB13	NA	+	-
8	IB17	NA	-	±
9	IB18	NA	-	-
10	IB21	NA	+	+
11	IB24	NA	±	±
12	IB25	NA	+	-
13	IB29	NA	±	+
14	IB32	NA	±	±
15	IB38	Zobell 2216E	+	-
16	IB39	Zobell 2216E	-	+
17	IB45	Zobell 2216E	±	-
18	IB46	Zobell 2216E	-	-
19	IB47	Zobell 2216E	+	+
20	IB48	Zobell 2216E	+	-
21	IF01	NA	±	±
22	IF02	NA	-	±
23	IF03	NA	+	±
24	IF04	NA	-	±
25	IF05	NA	-	±
26	IF06	NA	-	±
27	IF07	NA	+	±
28	IF08	NA	-	±
29	IF09	NA	-	±
30	IF10	NA	+	+
31	IF11	NA	±	±
32	IF12	NA	±	±
33	IF13	NA	+	+
34	IF14	NA	+	+
35	IF15	NA	+	±
36	IF16	NA	±	±
37	IF17	NA	±	±
38	IF18	NA	±	±

Note: IB: Bacterial, IF: Isolate Fungi, +: positive antibacterial activity, -: negative antibacterial activity, ±: has mist zoon.



A B

Figure 3. Antibacterial activity test of (1) bacterial isolates and (2) fungal isolates; (A) bacterial isolates, (B) pathogenic bacteria, (C) fungal isolates.

Table 2. Characterization Result of Bacterial Isolates

Test	Result	
	IB21	IB47
Morphology		
Gram staining	Positive	Negative
Motility	+	-
Shape of Bacterial	Bacilli	Coccus
Carbohydrate Fermentation		
D(+)-Glucose	+	+
Sucrose	+	+
D(+)-Mannitol	+	-
Lactose	-	-
Maltose	+	-
Enzyme Characteristic Test		
Urease	-	-
Catalase	+	+
Oxidase	+	+
Metabolite Products		
H_2S Production	-	-
Indole	-	+
Methyl Red	+	+
Voges Proskauer	-	-
Simmons citrate	+	-
O/F	+	+
Genus	*Corynebacterium*	*Neisseria*

Note: +: positive test/growth; -: negative test/growth

Table 3. Characterization Result of Fungi

Isolates	Colony Colour	Genera
IF01	Green	*Trichocladium* sp.
IF02	Brown	*Aspergillus* sp.
IF03	Light Brown	*Chaetomium* sp.
IF04	White	*Coprinus* sp.
IF05	Dark Brown	*Cladorrhinum* sp.
IF06	White Silver	*Aspergillus* sp.
IF07	Yellowish-brown	*Hymeochaete* sp.
IF08	Silver	*Rhizopus* sp.
IF09	Yellowish-brown	*Hymeochaete* sp.
IF10	White	*Tremella* sp.
IF11	White	*Zygorhynchus* sp.
IF12	White	*Aspergillus* sp.
IF13	Yellowish White	*Mucor* sp.
IF14	Purple	*Bjerkandera* sp.
IF15	Dark yellow	*Chaetomium* sp.
IF16	Silver	*Aspergillus* sp.
IF17	Gray	*Chaetomium* sp.
IF18	Dark green	*Aspergillus* sp.

4.4. Phytochemical Activity Test

IB21 isolate has alkaloids and saponin compound (Table 4). The IB47 isolate has alkaloids, saponins and phenolics. Secondary metabolites in bacteria are formed during the stationary phase and along with the change in energy sources from macromolecules to bioactivity [36][37].

Table 4. Phytochemical Activity Test Result

Phytochemical Test	Isolat	
	IB21	IB47
Flavonoid	-	-
Alkaloid		
Meyer	-	+
Dragendorff	+	+
Terpenoid	-	-
Steroid	-	-
Saponin	+	+
Phenolic	-	+

Note: +: positive, -: negative

5. CONCLUSIONS

The conclusions of this research were: 38 bacterial isolates and 18 fungal isolates were isolated from *C. racemosa*. Bacterial and fungal isolates had antibacterial activity against *S. aureus* and *E. coli*. Only two bacterial isolates namely IB21 and IB47 showed highest antibacterial activity. These two selected bacteria were characterized using biochemical test and they were identified as member of genus *Corynebacterium* and *Neisseria*, respectively. IB21 has positive in dragendorff (alkaloids) and saponins, IB47 has alkaloids, saponins and phenolics. Fungal isolates *Trichocladium, Aspergillus, Chaetomium, Coprinus, Cladorrhinum, Hymeochaete, Rhizopus, Tremella, Zygorhynchus, Mucor,* and *Bjerkandera.*

REFERENCES

[1] W. Merdekawati, A.B. Susanto, "Kandungan dan Komposisi Pigmen Rumput Laut Serta Potensinya Untuk Kesehatan", Squalen, 4(2): 41-47, 2009

[2] E. Ferawati, D.S. Widyartini, I. Insan, "Studi Komunitas Rumput Laut Pada Berbagai Substrat Di Perairan Pantai Permisan Kabupaten Cilacap", Scripta Biologica, 1(1): 57-62, 2014.

[3] N.P.S. Maharani, N. L. Watiniasih, A.P.W.K. Dewi, "Struktur Komunitas Makroalga di Pantai Geger dan Pantai Mengening Kabupaten Badung", Simbiosis, XI(1): 51-61, 2021.

[4] M.S.J. Sofiana, I. Safitri, I., S. Helena, Warsidah, "Phytochemical Screening, Total Phenolic Content And Antioxidant Activity of Tropical Brown Macroalgae Padina pavonica Hauck From Kabung Island, West Kalimantan", Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology, 17(1), 2021

[5] M.S.J. Sofiana, A.B. Aritonang, I. Safitri, S. Helena, S.I. Nurdiansyah, Risko, D. Fadly, "Proximate, Phytochemicals, Total Phenolic Content and Antioxidant Activity of Ethanolic Extract of Eucheuma spinosum Seaweed", Sys. Rev. Pharm., 11(8): 228-232, 2020

[6] W. F. Yap, V. Tay, S. H. Tan, Y. Y. Yow, and J. Chew, "Decoding Antioxidant and Antibacterial Potentials of Malaysian Green Seaweeds: Caulerpa racemosa and Caulerpa lentillifera," Antibiotics (Basel, Switzerland) vol. 8,3 152. 17 Sep. 2019

[7] P. Proksch, R.A. Edrata, R. Ebel, "Drugs from the sea-current status and microbiological implication", Appl. Microbiol. Biotechnol., 59: 125134, 2003.

[8] S. Rahmayanti, A. Massinai, S. Mashoreng, "Kepadatan Bakteri Simbion Rumput Laut (Eucheuma spinosum) yang Berasal dari Perairan Puntondo, Kabupaten Takalar, Sulawesi Selatan", Proceeding Simposium Nasional Kelautan dan Perikanan. 2019 Oct 21(6)

[9] R.P. Nugroho, A. Budiharjo, E. Kusdiyantini, "Bioprospeksi Bakteri Yang Berasosiasi Dengan Alga Hijau Halimeda macroloba, Caulerpa racemosa, dan Ulva sp Sebagai Penghasil Senyawa Antibakteri", Jurnal Akademika Biologi, 2(4), 8-15, 2013.

[10] K. Utami, N. Idiawati, M.S.J. Sofiana, "Aktivitas Antibakteri Bakteri Berasosiasi Caulerpa rasemosa dan Caulerpa taxifolia dari Perairan Singkawang". Jurnal Laut Khatulistiwa, 1(2), 55-60, 2018.

[11] Sartini, Suryadi, E.N. Zainuddin, "Isolation and Screening Fungal Symbiont in Green Alga Ulva reticulata as Candidate of Antibiotic Producer", Asian Jr. of Microbiol. Biotech. Env. Sc., 16(3): 475-478), 2014.

[12] D.A. Sumilat, R.A.J. Lintang. "Potensi antibakteri jamur Aspergillus nomius yang disolasi dari alga hijau Bornetella sp.", Jurnal Ilmiah Platax, 9(1), 2021

[13] A. Ismail, L. Ktari, M. Ahmed, H. Bolhuis, A. Boudabbous, J. L. Stal, M. S. Cretoiu, and M. E. l. Bour, "Microbial Activities of Bacteria Associated with the Brown Alga Padina pavonica," Front. Microbiol, 7:1072, 2016.

[14] A. C. Rahaweman, J. Pamungkas, H. Madduppa, C. Thoms, and K. Tarman, 2016, "Screening of Endophytic Fungi from Chlorophyta and Phaeophyta for Antibacterial Activity," J. IOP Conf. Series: Earth and Environmental Science, 1(31) : 1-7, 2016..

[15] W. Xie, P. Xu, W. Wang, and Q. Liu, "Preparation and antibacterial activity of a water-soluble chitosan derivative," Carbohydr. Res, 50:35–40, 2002.

[16] A. Ismail, 2010, "Isolasi dan Identifikasi Mikroba Endofit Penghasil Antibiotika pada Alga Merah *Eucheuma cottoni* Asal Rappoa Kabupaten Bantaeng", Universitas Islam Negeri Alauddin, Fakultas Ilmu Kesehatan, Jurusan Farmasi, Makassar, (Skripsi).

[17] Nurzakiyah, 2016, "Isolasi dan Identifikasi Molekuler Bakteri Endofit Caulerpa racemosa serta Aktivitas Antibakterinya Terhadap Staphylococcous aureus dan Methicillin Resistant Staphylococcous aureus (MRSA)," Universitas Islam Negeri Alauddin, Fakultas Sains dan Teknologi, Jurusan Biologi, Makassar, (Skripsi).

[18] R. Dewi, R. Nursanty, and C. Yulvizar, "The Effect of Storage Time on Total of Fungi in Kanji Pedah", J. Natural, 11(2) : 74-78, 2011

[19] D. Rizky, Warsidah, dan S. Helena, "Isolasi dan Identifikasi Fungi Berasosiasi Lamun Thalassia hemprichii dari Perairan Pulau Kabung", J. Laut Khat, 2(3) : 102-106, 2019.

[20] Yunaedi; Y. F. Victoria, L. Meylina, dan R. Rusli, "Isolasi dan Karakterisasi Jamur Endofit Akar Merung (Captosapelta tomentosa)", Prosiding Seminar Nasional Kefarmasian Ke-4, Samarinda: 20-21 Oktober 2016, Laboratorium Penelitian dan Pengembangan FARMAKA TROPIS Fakultas Farmasi Universitas Mulawarman Samarinda, Kalimantan Timur.

[21] C. G. Wang, J. Vlasa`k, and Y. C. Dai, "Phylogeny and Diversity of Bjerkandera (Polyporales, Basidiomycota), Including Four New Species from South America and Asia", J. Myco Keys, 79: 149-172. 2021.

[22] T. Watanabe, "Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species", CRC Press LLC, U.S.A. 2010.

[23] Y. Zhao, X. Z. Liu, and F. Y. Bai, "Four New Species of Tremella (Tremellales, Basidiomycota) Based on Morphology and DNA Sequence Data", J. Myco Keys, 47: 75-95, 2019.

[24] T. Nie, Y. Tian, S. L. Liu, J. Yang, and S. H. He, "Species of Hymenochaete (Hymenochaetales, Basidiomycota) on Bamboos from East Asia, with Descriptions of Two New Species", J. Myco Keys, 20: 51-65, 2017.

[25] M. T. Mardigan, J. M. Martiko, J. Parker, 1997, "Antibiotics Isolation and Characteriation", In:MT Mardigan (ed) Brock Biology of Microorganism, 8th ed, Prentice-Hall International Inc, New York, 1997.

[26] J. Flewelling, K. T. Ellsworth, J. Sanford, E. Forward, J. A. Johnson, and C. A. Gray, "Macroalga Endophytes from the Atlantic Coast of Canada: a potential source of antibiotic natural products", J. Microorganisms, 1: 175-187, 2013.

[27] Andriani, "Aktivitas Antibakterial Fungi Endofit Caulerpa racemosa Terhadap Bakteri Escherichia coli dan Staphylococcus aureus", Prosiding Seminar Nasional Mikrobiologi Kesehatan dan Lingkungan, Makassar: 29 Januari 2015, Jurusan Biologi, Fakultas Sains dan Teknologi, UIN Alauddin, Makassar, 2015.

[28] F. Ahamed, and M. Murugan, "Isolation and Characterization of Marine Endophytic Fungi from Seaweeds and Bioactivity of Their Crude Extracts", J. of Pure and Applied Microbiology, 13(3) : 1451-1460, 2019.

[29] Suryanarayanan, T.S.; Venkatachalam, A.; Thirunavukkarasu N.; Ravishankar, J.P.; Doble, M. and Geetha, V., 2010, Internal Mycobiota of Marine Macroalgae from the Tamilnadu Coast: distribution, diversity and biotechnological potential, J. Botanica Marina, 53: 457-468.

[30] A. Venkatachalam, R. M. B. Govinda, N. Thirunavukkarasu, and T. S. Suryanarayanan, "Endophytic Fungi of Marine Algae and Seagrasses: a novel source of chitin modifying enzymes", J. Mycosphere, 6(3) : 345-355, 2015.

[31] N. Lekshmi, M. D. Umar, M. Dhaneesha, J. Rojin, R. Ravinesh, and T. P. Sajeevan, "Endophytic Fungi Isolated from the Marine Sponges as a Source of Potential Bioactive Compounds", J. of Aquatic Biology & Fisheries, ISSN 2321-340X : 58-66, 2020.

[32] D. Handayani, N. Ananda, M. A. Artasasta, R. Ruslan, O. Fadriyanti, and T. E. Tallei, "Antimicrobial Activity Screening of Endophytic Fungi Extracts Isolated from Brown Algae Padina sp.", J. of Applied Pharmaceutical Science, 9(03) : 009-013, 2019.

[33] A. Massinai, A. Tahir, J. Jompa, dan A. Rantetondok, "Bakteri Berasosiasi di Karang Batu (Skleractinia) yang Terinfeksi Penyakit Tumor (Growth Anomalies) yang Berasal dari Pulau Salemo Kabupaten Pangkep, Spermonde", 3(1): 7-12, 2017.

[34] B. E. Pratiwi, "Isolasi Dan Skrinning Fitokimia Bakteri Endofit Dari Daun Rambutan (Nephelium lappaceum L.) Yang Berpotensi Sebagai Antibakteri", Porgram Studi Farmasi, Fakultas Kedokteran Dan Ilmu Kesehatan, Jakarta, (Skripsi), 2015.

[35] B. Ruiz, A. Chavez, A. Forero, Y. Garcia-Huante, A. Romero, and M. Sanchez, "Production of microbial secondary metabolites: Regulation by the carbon source". Crit. Rev. Microbiol. 36", 146–167, 2010.

[36] D. Iqlima, P. Ardiningsih, dan M. A. Wibowo, "Aktivitas antibakteri isolat bakteri endofit B2D dari batang tanaman yakon (Smallanthus sonchifolius (Poepp. & Endl.) H. Rob.) terhadap bakteri Staphylococcus aureus dan Salmonella thypimurium", Jurnal Kimia Khatulistiwa, 7(1), 2017.

[37] Masriani, D. Fadly, and B. Bohari, "α-Glucosidase Inhibitory Activity of Ethanol Extract Obtained from Dillenia suffruticosa and Pycnarrhena cauliflora", J. Glob. Pharma Technol, 12, 881–887, 2020.

[38] M. Ventura, C. Canchaya, A. Tauch, G. Chandra, G. F. Fitzgerald, K. F. Chater, and D. S. Van, "Genomics of Actinobacteria: tracing the evolutionary history of an ancient phylum", Microbiol. Mol. Biol. Rev, 71, 495–548, 2007.