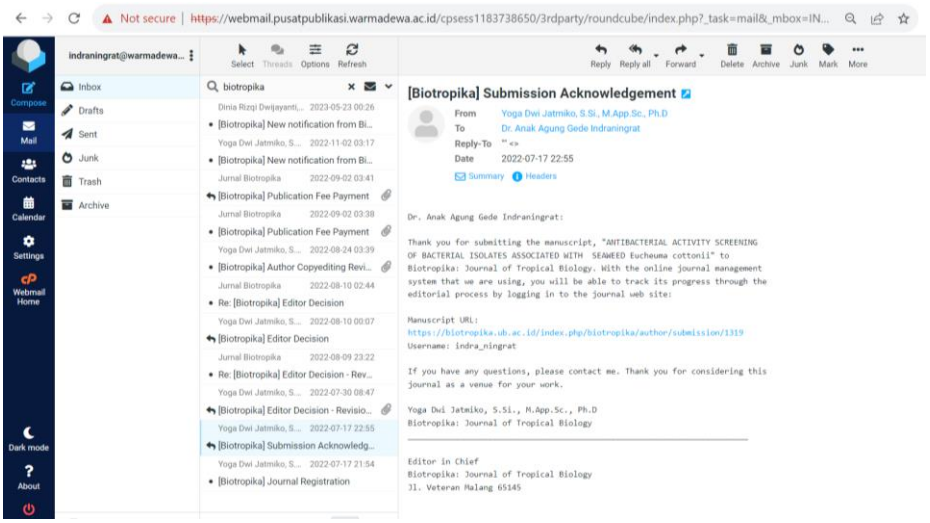
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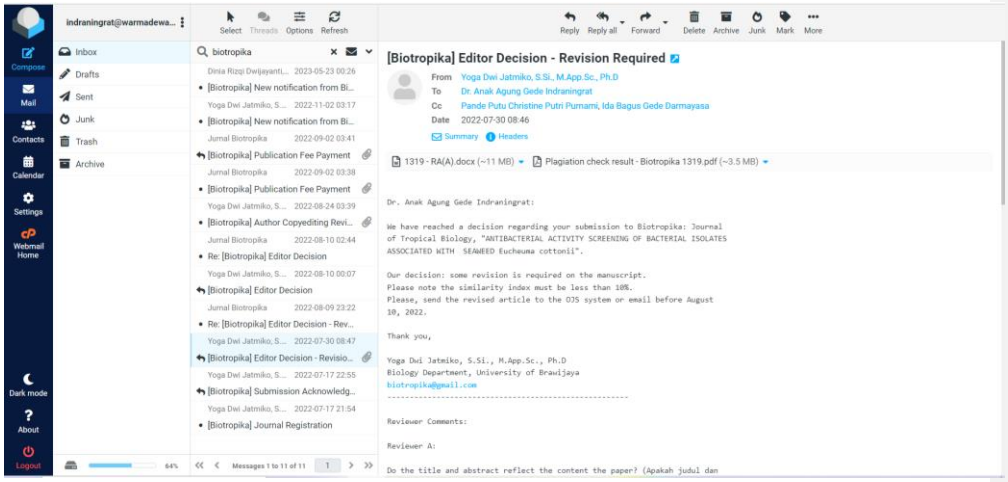
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ANTIBACTERIAL ACTIVITY SCREENING OF BACTERIAL ISOLATES ASSOCIATED WITH SEAWEED *Eucheuma cottonii*

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SKRINING AKTIVITAS ANTIBAKTERI ISOLAT BAKTERI YANG BERASOSIASI DENGAN RUMPUT LAUT Eucheuma cottonii

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ABSTRACT

Seaweeds like other marine organisms develop a strong association with prokaryotic communities, especially bacteria. Many of these associated bacteria play an important role in synthesizing secondary metabolites which are beneficial for their host, especially against predation or infection. In Indonesia, Eucheuma cottonii is one of the seaweed species that has been cultivated widely. However, only limited study has been reported on bacterial isolates and their antibacterial activity from E. cottonii. Bacterial isolates from E. cottonii were cultivated in six agar media namely Zobell Marine Agar, Nutrient Agar, Plate Count Agar, ISP-1, ISP-2, and Starch-M protein agar. Cultivation of bacterial isolates yielded 23 isolates with nine bacterial isolates were classified as Gram-positive bacteria and 14 isolates were grouped among Gram-negative bacteria. All the bacterial isolates were screened for their antibacterial activity against six bacterial indicator strains namely Staphylococcus aureus ATCC 25923, Streptococcus mutans FNCC 0405, Escherichia coli ATCC 25922, and Klebsiella pneumoniae ATCC 700603 using perpendicular streak and agar block method. Six out of 23 bacterial isolates displayed antibacterial activity against at least one of the bacterial indicator strains. The bacterial isolate ISP1RL4 showed the highest antibacterial activity with average inhibition of > 20 mm against all bacterial indicators. Overall, our result indicated the potential of bacterial isolates associated with E. cottonii as an antibacterial producer.

Keywords: *antibacterial, Eucheuma cottonii, isolation, seaweed*

ABSTRAK

Rumput laut seperti organisme laut lainnya membangun asosiasi yang kuat dengan komunitas prokariotik khususnya bakteri. Banyaknya bakteri yang berasosiasi ini berperan penting dalam mensintesis senyawa metobolit sekunder yang bermanfaat bagi inangnya, khususnya terhadap predasi atau infeksi. Di Indonesia, Eucheuma cottonii ialah salah satu spesies rumput laut yang telah dibudidayakan secara luas. Namun, sejauh ini masih terbatas penelitian yang melaporkan isolat bakteri dan aktivitas antibakterinya dari E. cottonii. Bakteri yang berasosiasi dengan E. cottonii dikultivasi pada enam media agar yaitu Zobell Marine Agar, Nutrient Agar, Plate Count Agar, ISP-1, ISP-2, dan Strach-M Protein agar. Hasil isolasi bakteri mendapatkan 23 isolat dengan sembilan isolat bakteri tergolong bakteri Gram positif dan 14 isolat tergolong bakteri Gram negatif. Seluruh isolat bakteri diskrining aktivitas antibakterinya terhadap enam jenis bakteri indicator yaitu Staphylococcus aureus ATCC 25923, Streptococcus mutans FNCC 0405, Escherichia coli ATCC 25922 dan Klebsiella pneumoniae ATCC 700603 menggunakan metode goresan tegak lurus dan blok agar. Enam dari 23 isolat bakteri menunjukkan aktivitas antibakteri terhadap setidaknya satu jenis bakteri indikator. Isolat bakteri ISP1RL4 menampilkan aktivitas antibakteri tertinggi dengan rata-rata zona hambat > 20 mm terhadap seluruh bakteri indikator. Secara keseluruhan, hasil kami menunjukkan potensi isolat bakteri yang berasosiasi dengan E. cottonii sebagai penghasil antibakteri.

How to cite:

INTRODUCTION

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Infection by pathogenic bacteria is still the leading cause of death globally, especially in developing countries [1]. The incidence of infection diseases is estimated in the ranged between 3.5% and 12% in high income countries, whereas it varies between 5.7% and 19.1% in middle and low-income countries [2]. Developing countries are less able to prevent infection. Countries classified as low- and middle-income countries (LMICs) — defined as those with a per capita GNI of less than $4,125 — were more likely to experience clean water shortages and sanitation and hygiene problems [3]. Not only are more people affected by bacterial pathogens in low- and middle-income countries, but those who are infected tend to be more susceptible [3]. The high incidence of infectious diseases worldwide is also influenced by the increasing rate of antimicrobial resistance [2,3]. The existence of antibiotic resistance in some pathogenic bacteria causes the use of antibiotics or other antimicrobial drugs to be ineffective and infections to become increasingly difficult or impossible to treat [4]. Therefore, efforts to explore natural sources of antibacterial compounds that have better capabilities than drugs already circulating in the market must continue to be carried out to overcome the increasing number of infectious diseases caused by bacteria. The search for new antibiotic compounds originating from rare and natural sources will yield useful clues in the

identification of new drugs against the increasing resistance of pathogenic bacteria [5].

So far, efforts to search for new antibacterial compounds have been focused on terrestrial ecosystems. However, the discovery rate of antibacterial compounds from terrestrial sources is often limited by the discovery of existing compounds (dereplication) [6]. Therefore, current studies have been focused to search for novel antibacterial compounds from marine ecosystem since they are rather unexplored and offer a source of unique secondary metabolites [7]. Several antibacterial active compounds from marine organisms have been found, such as in sponge-associated bacteria with *Streptomyces*, the most commonly found genus as the producer of sponge bacteria-derivative compounds [8]. *Streptomyces* HB202, isolated from the sponge *Halichondria panicea*, has been shown to produce three antibacterial substances, such as mayamycin, streptophenazine G, and K, which are mainly active against Gram-positive pathogens [9]. Peptide compounds isolated from marine invertebrates of the phyla Cnidaria, Molluscs, Annelids, Arthropods, and Echinoderms also showed antibacterial properties [10]. Myticusin-1 is a cysteine-rich peptide, which has been successfully isolated from mussel *Mytilus coruscus* that can inhibit the growth of Gram-positive bacteria, such as *Staphylococcus aureus* (MIC < 5 mM), and Gram-negative, such as *Escherichia. coli* (MIC > 10 mM) [11].

Among marine organisms, seaweeds are one of the producers for secondary metabolites including antibacterial compounds. Seaweed was reported to contain halogenated compounds, sterols, heterocyclics, and phenolic compounds in seaweed that showed antibacterial effects against Gram-positive and negative bacteria [12]. Seaweed also has fucoxanthin pigment which has been shown to have effective antibacterial activity against several gram-positive bacteria such as *Streptococcus agalactiae*, *S. epidermidis*, and *S. aureus* [13]. Polysaccharides, such as fucoidan and laminarin, in seaweed, have been successfully used in drug administration as oral antibiotics to inhibit the growth of *S. aureus* and *E. coli* [14]. Seaweed naturally builds beneficial associations especially with bacteria by providing a suitable substrate for bacterial colonization and also secretes various organic substances that function as nutrients for bacterial propagation [15]. On the other hand, bacteria associated with seaweed actively synthesize various secondary metabolites to protect the host from infection or predation [16]. Seaweeds are known to host various species of Actinobacteria, both epiphytic and endophytic. About 80% of antibiotics are mainly produced by Actinobacteria, especially the genera Streptomyces and Micromonospora [17]. Significant efforts have been focused on the isolation of novel marine actinobacteria to develop drug discovery especially antibiotics [18]. Several studies have shown that actinobacteria isolated from seaweed are capable of producing bioactive compounds, including antibiotics [19, 20] and showed antibacterial activity against several pathogenic bacteria, such as *Proteus* sp., *Enterobacter* sp. [21], *Vibrio alginolyticus* [18], and *S. aureus* [22]. Apart from actinobacterial isolates, other bacteria that associated with seaweed has also been reported antibacterial activity such as *Pseudomonas* sp, *Stenotrophomonas* sp, *Vibrio* sp, *Altomonas* sp, *Shewanella* sp, and *Bacillus* sp[23].

One of the most common types of seaweed found in Indonesian seawater is *Eucheuma cottonii*, which is cultivated for the food and non-food product industry [24]. The association of bacteria with seaweed *E. cottonii* has been shown to provide antibacterial activity [25, 26]. A study has reported that two bacterial isolates were isolated from *E. cottonii* seaweed in the North Galesong Sea, Takalar, South Sulawesi, namely *Aeromonas* sp. and *Klebsiella* sp. [27]. A follow up study reported that the isolate *Aeromonas* sp. was the only bacterial species with antibacterial activity against *S. aureus* and *E. coli* [25]. Another study on the cultivation of bacterial isolates from seaweed *E. cottonii* from Bangsal beach, Lombok, West Nusa Tenggara reported two Actinomycetes isolate namely RL 6 and RL 12 have inhibited the growth of *S. aureus* and *E. coli* with zone of inhibition of >30 mm [26].

Studies on the isolation, characterization, and screening of the antibacterial activity of the bacterial community associated with *E. cottonii* seaweed from the coastal waters of Bali are rather limited. To date, studies on *E. cottonii* seaweed in coastal waters in Bali were more focused to analyze growth rates and potential for aquaculture development [28, 29]. Therefore, this study was aimed to identify morphology of bacterial isolates associated with seaweed *E. cottonii* and to screen for antibacterial activities of these isolates against Gram-positive and Gram-negative bacterial indicator strains. It is expected that the outcome of this research will lead for a bacterial isolate with strong antibacterial activity.

METHODS

Sampling of *E. cottonii*. This research was conducted from November 2021 to March 2022 at the Laboratory Agriculture, Faculty of Agriculture, Warmadewa University. Samples of *E. cottonii* were taken from the beach in Patas Singaraja Village, Gerokgak District, Buleleng Regency, Bali Province (8°11'09.6"S, 114°48'48.6"E). Approximately 100 g of *E. cottonii* was taken aseptically using sterile gloves and a knife. The obtained samples were stored in a sterile

Falcon tube and closed tightly. In addition, 1 L of seawater was taken from the sampling location and stored in a sterile glass bottle (Durant). Samples of *E. cottonii* and seawater were stored in a cool box that already contained an ice pack. Subsequently, samples were transported to the laboratory and stored at 4 °C until further testing.

Cultivation conditions. Samples of *E. cottonii* seaweed were washed with sterile artificial seawater three times, weighed 10 grams, and cut into pieces to ensure that all thallus parts, including the interior and exterior, were evenly mixed. The sample was homogenized by pounding it under sterile conditions with mortar and pastel and then adding 25 mL of sterile artificial seawater. A total of 1 mL of *E. cottonii* seaweed suspension was diluted in stages in a test tube containing 9 mL of sterile artificial seawater (10^{-1} to 10^{-6}). Each dilution of 10^{-3} to 10^{-5} was taken as much as 200 µL to be planted on a predetermined cultivation media and spread using a sterile cotton swab. The following six cultivation media were used which three of them were aimed for non-actinobacterial species namely Zobell Marine Agar (Himedia), Nutrient Agar (Himedia), and Plate Count Agar (Oxoid). In addition, three other media were aimed specifically for actinobacterial isolates namely ISP-1 (5.0 grams/L peptone, 3.0 grams/L yeast extract), ISP-2 (4.0 grams/L yeast extract, 10 grams/L malt extract, 4 grams/L dextrose) and Starch-M protein agar (Himedia). Four media namely NA, PCA, ISP-1, and ISP-2 were dissolved in artificial seawater (33 grams/L). All media were 20 grams/L of bacto agar (Himedia) to produce solid media. After autoclaving, ISP-1, ISP-2, and Starch M-protein agar media were supplemented with Nystatin (800 µL) and nalidixic acid (200 µL). Petri dishes containing agar media and samples were then wrapped in parafilm and incubated upside down in an incubator at 28 °C. Periodic observations were made every 3 days for a maximum of two weeks to count the number of colonies that appeared on each agar media. Individual bacteria colonies with different morphologies that grow on media were picked and characterized according to the colony morphology code (CMC) criteria [30] (Figure 1). Non-Actinobacterial isolates were purified on Zobell marine agar media, while Actinobacteria candidate isolates were purified on ISP-2 agar. The selected colonies with different morphology were colorized based on Gram staining procedure [31] and catalase test [32]. Subsequently, cell morphology was observed under light microscope (Leica DM750).

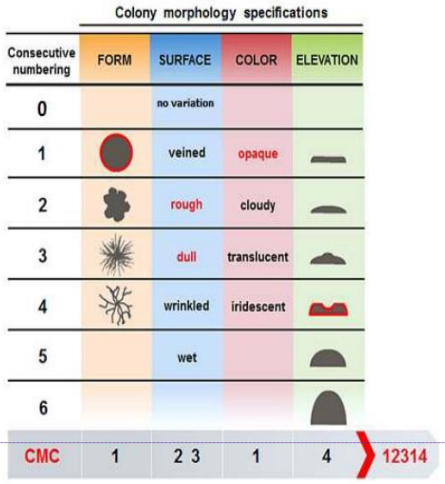

Figure 1. Coding of bacterial colony morphology based on the CMC method [30].

Screening of antibacterial activity. Antibacterial activity screening was carried out using two methods, namely perpendicular streak [33] for non-actinobacterial isolates and agar block methods [34, 35, 36] for actinobacterial candidates. Briefly, bacterial isolates were streaked on LB agar perpendicularly with distance of 8 cm and subsequently the agar plate were incubated for 24 hours at 28 °C until colonies were formed on agar. Furthermore, four bacterial indicator strains namely *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* FNCC 0405 *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 were streaked horizontally from the tested isolates with distance of 3 cm (Figure 2). Subsequently, agar plates were incubated for 24 hours at 37 °C and the length of the inhibition zone (in mm) formed on each test bacteria was measured [33]. The zone of inhibition was calculated based on the distance of bacterial growth by bacterial isolates to each of the tested bacteria.

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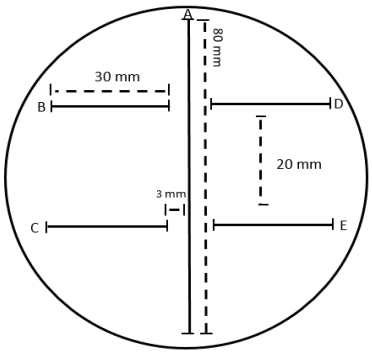

Figure 2. Streaking pattern for screening using the perpendicular streak method [30]. A. Isolates of test bacteria, B. *Staphylococcus aureus* ATCC 25923, C. *Streptococcus mutans* FNCC 0405, D. *Escherichia coli* ATCC 25922, E. *Klebsiella pneumoniae* ATCC 700603.

Agar block method started by refreshing the four bacterial indicator strains in LB broth. Furthermore, 200 µL of suspension for each test bacterium with an optical density (OD) of 0,5 was spread with a sterile cotton swab on LB agar. Subsequently, the actinobacteria isolates that had been purified on ISP-2 agar media with a minimum age of 10 days were cut into blocks of 1 x 1 cm using a sterile scalpel. Block pieces for actinobacterial isolates were then placed in each petri dish containing the test bacteria and incubated at 37 ºC for 48 hours. The antibacterial activity against bacterial indicator strains was observed by the formation of a clear zone around the agar block [35]. The diameter of clear zone was measured using a digital caliper.

RESULTS AND DISCUSSION

Morphological characteristics of bacterial isolates. A total of 23 bacterial isolates were isolated from *E. cottonii* which further can be divided into 11 isolates from general media and 12 isolates from specific media (Table 1A and Table 1B). Majority of isolates obtained from general media had an irregular shape, a dull surface, an opaque color, and a flat elevation. Meanwhile, bacterial isolates from specific media have more diverse appearances such as irregular shapes, and circular. Moreover, their surface tended to be rough and dull, color was mostly opaque and elevations that vary from flat, raised, umbonate, to crateriform. Cell wall composition of these isolates were dominantly Gram-negative with 14 bacterial isolates (70%) and the remaining were Gram-positive bacteria with 9 bacterial isolates (30%). Furthermore, 20 bacterial isolates (87%) have positive catalase activity and three bacterial isolates (13%) have negative catalase.

Twenty-three isolates of bacteria have individual characteristics so they are considered to come from different types of bacteria. Apart from the obtained number of colonies that vary, it cannot be denied that not all bacteria associated with *E. cottonii* can be isolated. Naturally, not all bacterial isolates can be cultured under the standard culture system carried out for the laboratory scale. This is influenced by factors of inadequate growth conditions, low growth rates, the need for metabolites produced by other bacteria, and the presence of inactive cells [37] so it is estimated that only about 1 in 100 microbes can be cultured, which refers to phenomenon of "the great plate count anomaly" [38]. To date, most of the available information on bacterial and seaweed interactions has been obtained from culture-based studies, and it is estimated that only 1 to 10% of associated bacteria can be cultured [39].

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Table 1. Morphology of bacterial isolates according to CMC, Gram stain, and catalase

A. Morphology of Bacterial Isolates from *E. cottonii* on General Media

Isolate code	Morphology of isolate				Type of Gram	Cell Shape	Catalase
	Form	Surface	Colour	Elevation			
ZRL1	2 (Irregular)	3 (Dull)	1 (Opaque)	1 (Flat)	-	Coccus	+
ZRL2	1 (Circular)	3 (Dull)	3 (Translucent)	2 (Raised)	-	Coccus	+
NRL1	1 (Circular)	3 (Dull)	1 (Opaque)	1 (Flat)	-	Coccus	-
NRL2	1 (Circular)	3 (Dull)	1 (Opaque)	1 (Flat)	+	Coccus	+
NRL3	1 (Circular)	1 (Veined)	1 (Opaque)	1 (Flat)	+	Coccus	+
NRL4	2 (Irregular)	1 (Veined)	1 (Opaque)	1 (Flat)	-	Coccus	-
PCARL1	2 (Irregular)	3 (Dull)	1 (Opaque)	1 (Flat)	+	Coccus	+
PCARL2	2 (Irregular)	3 (Dull)	3 (Translucent)	1 (Flat)	-	Bacilli	+
PCARL3	2 (Irregular)	24 (Rough and wrinkled)	3 (Translucent)	1 (Flat)	-	Bacilli	+
PCARL4	1 (Circular)	3 (Dull)	1 (Opaque)	1 (Flat)	+	Bacilli	+
PCARL5	2 (Irregular)	23 (Rough and dull)	1 (Opaque)	1 (Flat)	-	Coccus	+

B. Morphology of Bacterial Isolates from *E. cottonii* on Specific Media

Isolate code	Morphology of isolate				Type of Gram	Cell Shape	Catalase
	Form	Surface	Colour	Elevation			
ISP1RL1	2 (Irregular)	34 (Dull and wrinkled)	1 (Opaque)	4 (Crateriform)	+	Bacillus	+
ISP1RL2	1 (Circular)	2 (Rough)	1 (Opaque)	2 (Raised)	-	Bacillus	-
ISP1RL3	1 (Circular)	3 (Dull)	3 (Translucent)	1 (Flat)	-	Bacillus	-
ISP1RL4	1 (Circular)	34 (Dull and wrinkled)	4 (Yellow)	2 (Raised)	+	Bacillus	+
ISP1RL5	2 (Irregular)	3 (Dull)	1 (Opaque)	1 (Flat)	-	Bacillus	-
ISP1RL6	2 (Irregular)	23 (Rough and dull)	1 (Opaque)	1 (Flat)	-	Bacillus	-
ISP2RL1	2 (Irregular)	23 (Rough and dull)	4 (Yellow)	3 (Umbonate0	-	Bacillus	-
ISP2RL2	1 (Circular)	3 (Dull)	1 (Opaque)	2 (Raised)	-	Bacillus	-
ISP2RL3	3 (Filamentous)	23 (Rough and dull)	1 (Opaque)	1 (Flat)	+	Bacillus	+
SMPRL1	2 (Irregular)	34 (Dull and wrinkled)	1 (Opaque)	4 (Crateriform)	+	Bacillus	+
SMPRL2	2 (Irregular)	3 (Dull)	4 (Yellow)	2 (Raised)	+	Bacillus	+
SMPRL3	2 (Irregular)	23 (Rough and dull)	1 (Opaque)	2 (Raised)	-	Bacillus	-

Description: ISP1RL: ISP-1 Agar Media, ISP2: ISP-2 Agar Media, SMPRL: Starch-M Protein Agar Media, +: Positive, -: Negative.

Evaluation of the antibacterial activity of bacterial isolates associated with *E. cottonii.* Antibacterial activity screening of isolates associated with *E. cottonii* became the main focus of this study to obtain potential isolates that could produce antibacterial compounds. Screening carried out to find new antibacterial compounds must be simple, fast, repeatable, and inexpensive [40]. The antibacterial activity screening method used consisted of two methods, namely the perpendicular streak method and the agar block method. These methods were chosen because the procedure is fast, easy, and efficient to select isolates with antibacterial activity without the need for an extraction step [41].

~~Based on the results of the study, the antibacterial activity with the perpendicular streak on 11 common bacterial isolates against~~

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~~the four tested bacteria can be seen in Table 2A. The results of the antibacterial activity test using the agar block method on 12 isolates can be seen in Table 2B.~~

Table 2. Antibacterial Activity of Bacteria Isolated from *E. cottonii*

A. Zone of Inhibition of Bacterial Isolates from General Media

No	Sample code	Zone of inhibition (mm)			
		S. aureus	*S. mutans*	*E. coli*	*K. pneu*
1	ZRL1	-	-	-	-
2	ZRL2	-	-	-	-
3	NRL1	-	-	-	-
4	NRL2	-	-	-	-
5	NRL3	-	-	-	-
6	NRL4	-	-	-	-
7	PCARL1	-	-	-	-
8	PCARL2	-	-	-	-
9	PCARL3	-	-	-	-
10	PCARL4	-	-	-	-
11	PCARL5	-	-	-	-

B. Zone of Inhibition of Bacterial Isolates from Specific Media

No	Sample code	Zone of Inhibition (mm)			
		S. aureus	*S. mutans*	*E. coli*	*K. pneu*
1	ISP1RL1	-	-	-	-
2	ISP1RL2	-	33,9	31,6	23,8
3	ISP1RL3	19,9	33,4	29,9	22
4	ISP1RL4	21,7	35,4	27,6	31,1
5	ISP1RL5	-	33,7	29,4	2
6	ISP1RL6	-	31,1	29	4
7	ISP2RL1	-	-	-	-
8	ISP2RL2	-	-	-	-
9	ISP2RL3	-	-	-	-
10	SMPRL1	-	-	-	-
11	SMPRL2	29,4	-	-	-
12	SMPRL3	-	-	-	-

Description: *S. aureus* : *Staphylococcus aureus* ATCC 25923, and *S. mutans* : *Streptococcus mutans* FNCC 0405. *E. coli* : *Escherichia coli* ATCC 25922, *K. pneumoniae* : *Klebsiella pneumoniae* ATCC 700603.

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All bacterial isolates on general media could not provide antibacterial activity against the four test bacteria which were indicated by the absence of an inhibition zone formed in the streaked area of the test bacteria (Table 2A). Meanwhile, six bacterial isolates were obtained from specific media that could provide antibacterial activity against the test bacteria, namely isolates with codes ISP1RL2, ISP1RL3, ISP1RL4, ISP1RL5, ISP1RL6, and SMPRL2 (Table 2B). Antibacterial activity was classified into four categories, namely weak (<5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm) [42]. These six isolates were able to inhibit at least one isolate of the test bacteria and showed very strong antibacterial activity with a mean inhibition zone of more than 20 mm, except for isolates ISP1RL5 and ISP1RL6 which had a weak inhibition for *K. pneumoniae*. The highest inhibition zone for each test bacteria can be seen in Figure 3.

Differences in ability to produce clear zones may depend on secondary metabolites produced by each bacterial isolate. Secondary metabolite componds are very sensitive to changes in environmental and cultural conditions [43]. Therefore, the *in vitro* production of most antibiotics depends on the composition of the medium in which the production microorganisms are grown [43]. Based on this, it can be assumed that the nutritional composition of LB agar media used in the antibacterial activity test cannot accommodate optimal bacterial growth, so that the bacteria cannot produce their secondary metabolites causing no inhibition zones to be formed.

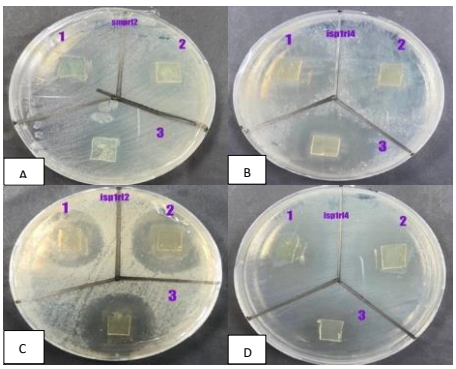

Figure 3. Inhibition of *E. cottonii* seaweed isolates against test bacteria. A. ISP1RL2 isolate had the highest zone of inhibition against *S. aureus* test bacteria at 29,4 mm; B. ISP1RL4 isolate had the highest inhibition zone against the test bacteria *S. mutans* at 35,4 mm; C. ISP1RL2 isolate had the highest zone of inhibition against *E. coli* test bacteria at 31,6 mm; D. ISP1RL4 isolate had the highest zone of inhibition against the test bacteria *K. pneumoniae* at 31,1 mm.
Description: 1 = the first replicate ; 2 = second replicate; 3 = third replicate.

The inhibition zones formed on each test bacteria varied greatly with the range of inhibition zones ranging from 20–30 mm, 31–34 mm, 27–32 mm 2–32 mm, respectively for *S. aureus*, *S. mutans*, *E. coli*, and *K. pneumoniae*. In general, these active isolates were more active against Gram-positive bacteria *S. aureus* and *S. mutans* than the Gram-negative bacteria *E. coli* and *K. pneumoniae*. Such discrepancies of antibacterial activity could happen because Gram-negative bacteria have a better defense system against antibacterial compounds than Gram-positive bacteria, which is influenced by differences in the cell wall components between the two group of bacteria [44]. In addition, Gram-negative bacteria also have an outer membrane, that composed mainly of lipopolysaccharide which covers 90% of the cell surface and functions as an additional protection system, so that Gram-negative bacteria are more resistant against antibacterial compounds [44, 45].

Based on the results of macroscopic and microscopic identification, and the presence of antibacterial activity, two candidate isolates of bacteria were chosen, namely isolates ISP1RL4 and SMPRL2 (Figure 4) with characteristics resembles Actinobacteria. These two isolates have Gram-positive, rod-shaped cells, have antibacterial activity commonly found in the Actinobacteria group [46], aerial mycelium with gray-yellow pigmentation on ISP1RL4, and the presence of pale-yellow aerial spores on SMPRL2, and slow growth [47]. Of the two isolates, ISP1RL4 was considered as the best

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actinobacterial candidate with the highest range of inhibition for all test bacteria.

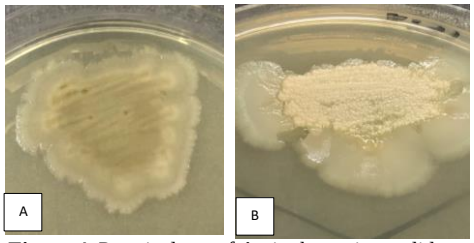

Figure 4. Pure isolates of Actinobacteria candidates (a) ISP1RL isolate; (b) SMPRL2 isolate.

The antibacterial screening of the six bacterial isolates from *E. cottonii*: ISP1RL2, ISP1RL3, ISP1RL4, ISP1RL5, ISP1RL6, and SMPRL2 show specificity of each isolate against each type of Gram-positive or Gram-negative bacteria. Further research needs to be focused to explore bioactivity of these isolates against multidrug resistant bacteria and also against other microbes such as parasitic fungi dan parasites.

CONCLUSION

In conclusion, we isolated 23 bacterial isolates from seaweed *E. cottonii*. Six bacterial isolates namely ISP1RL2, ISP1RL3, ISP1RL4, ISP1RL5, ISP1RL6, and SMPRL2 displayed antibacterial activity against at least one of bacterial indicator strains. Among the six isolates, ISP1RL4 showed the highest antibacterial activities. Further research will be focused to sequence the six isolates by amplifying 16S rRNA gene fragment.

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ACKNOWLEDGEMENTS

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REFERENCES

[1] Sarmah, P., Dan, M. M., Adapa, D., & TK, S. (2018). A review on common pathogenic microorganisms and their impact on human health. *Electronic Journal of Biology*, 14(1), 50–58.

[2] Khan, A. H., Baig, F. K., & Mehbob, R. (2018). Nosocomial infections: Epidemiology, prevention, control, and surveillance. *Asian Pacific Journal of Tropical Biomedicine*, 7(5), 478-482. https://doi.org/10.1016/j.apjtb.2017.01.019

[3] Nagel, T. E., Chan, B. K., De Vos, D., El-Shibiny, A., Kang'ethe, E. K., Makumi, A., & Pirnay, J-P. (2016) The Developing World Urgently Needs Phages to Combat Pathogenic Bacteria. *Front. Microbiol.* 7:882. https://doi.org/10.3389/fmicb.2016.00882.

[4] WHO (2020) *Antimicrobial resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

[5] Ali, S. M., Siddiqui, R., & Khan, N. A. (2018) Antimicrobial discovery from natural and unusual sources. *Journal of Pharmacy and Pharmacology*, 70(10):, 1287–1300.

[6] Cox, G., Sieron, A., King, A. M., De Pascale, G., Pawlowski, A. C., Koteva, K., & Wright,

G. D. (2017). A Common Platform for Antibiotic Dereplication and Adjuvant Discovery. *Cell Chemical Biology*, *24*(1), 98–109. https://doi.org/10.1016/j.chembiol.2016.11.011.

[7] Hai, Y., Wei, M. Y., Wang, C. Y., Gu, Y. C., & Shao, C. L (2021) The intriguing chemistry and biology of sulfur-containing natural products from marine microorganisms (1987–2020). *Marine Life Science and Technology*, *3*(4), 488–518. https://doi.org/10.1007/s42995-021-00101-2

[8] Indraningrat, A. A. G., Smidt, H., & Sipkema, D. (2016). Bioprospecting Sponge-Associated Microbes for Antimicrobials Compounds. *Mar. Drugs*, 14(5), 87. https://doi.org/10.3390/md14050087.

[9] Schneemann, I., Kajahn, I., Ohlendorf, B.; Zinecker, H., Erhard, A., Nagel, K., Wiese, J., Imhoff, J.F. Mayamycin, a cytotoxic polyketide from a *Streptomyces* strain isolated from the marine sponge *Halichondria panicea*. *J. Natl. Prod.*, 73, 1309–1312. https://doi.org/10.1021/np100135.

[10] Macedo, M. W. F. S., Cunha, N. B., Carneiro, J. A., Costa, R. A., Alencar, S. A., Cardoso, M. H., Franco, O. L., & Dias, S. C. (2021). Marine Organisms as a Rich Source of Biologically Active Peptides. *Front. Mar. Sci.*, 8, 667764. https://doi.org/10.3389/fmars.2021.667764.

[11] Liao, Z., Wang, X., Liu, H., Fan, M., Sun, J., and Shen, W. (2013). Molecular characterization of a novel antimicrobial peptide from Mytilus coruscus. Fish Shellfish Immunol. 34, 610–616. https://doi.org/10.1016/j.fsi.2012.11.030.

[12] Salehi, B., Sharifi-Rad, J., Seca, A. M. L., Pinto, D. C. G. A., Michalak, I., Trincone, A., Mishra, A. P., Nigam, M., Zam, W., & Martins, N (2019) Current trends on seaweeds: Looking at chemical composition, phytopharmacology, and cosmetic applications. *Molecules*, 24(22).

[13] Karpinksi, T. M., & Adamczak, A. (2019). Fucoxanthin—An Antibacterial Carotenoid. *Antioxidants (Basel)*, 8(8), 239. https://doi.org/ 10.3390/antiox8080239

[14] Shannon, E., & Abu-Ghannam, N. (2016). Antibacterial Derivatives of Marine Algae: An Overview of Pharmacological Mechanisms and Applications. *Mar. Drugs*, 14(40: 81. https://doi.org/10.3390%2Fmd14040081.

[15] Singh, R. P., & Reddy, C. R. K (2014) Seaweed-microbial interactions: Key functions of seaweed-associated bacteria. *FEMS Microbiology Ecology*, 88(2):, 213–230.

[16] Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., & Thomas, T (2013) The seaweed holobiont: Understanding seaweed-bacteria interactions. *FEMS Microbiology Reviews*, 37(3):, 462–476.

[17] Anandan, R., Dharumadurai, D., & Manogaran, G. P. (2016). An Introduction to Actinobacteria. *Actinobacteria - Basics and Biotechnological Apllications*. IntechOpen.

[18] Ulfah, M., Kasanah, N., & Handayani, N. S. N (2018) Bioactivity and genetic screening of marine actinobacteria associated with red algae *Gelidiella acero*sa. *Indonesian Journal of Biotechnology*, *22*(1), 13–21. https://doi.org/10.22146/ijbiotech.25920

[19] Braña, A. F., Fiedler, H. P., Nava, H., González, V., Sarmiento-Vizcaíno, A., Molina, A., Acuña, J. L., García, L. A., & Blanco, G (2015) Two Streptomyces Species Producing Antibiotic, Antitumor, and Anti-Inflammatory Compounds Are Widespread Among Intertidal Macroalgae and Deep-Sea Coral Reef Invertebrates from the Central Cantabrian Sea. *Microbial Ecology*, *69*(3), 512–524. https://doi.org/10.1007/s00248-014-0508-0

[20] Uzair, B., Menaa, F., Khan, B. A., Mohammad, F. V., Ahmad, V. U., Djeribi, R., & Menaa, B (2018) Isolation, purification, structural elucidation and antimicrobial activities of kocumarin, a novel antibiotic isolated from actinobacterium *Kocuria marin*a CMG S2 associated with the brown seaweed *Pelvetia canaliculata. Microbiological Research*, *206*, 186–197.https://doi.org/10.1016/j.micres.2017.10.007

[21] Negara, B. F. S. P., Riyanti, ., Marhaeni, B., & Kusuma, A. B (2016) Antibacterial activity of Actinomycetes symbiont with seaweeds: a prosperous agent of animal antibacterial. *Aceh Journal of Animal Science*, *1*(2), 45–49. https://doi.org/10.13170/ajas.1.2.4475

[22] Girão, M., Ribeiro, I., Ribeiro, T., Azevedo, I. C., Pereira, F., Urbatzka, R., Leão, P. N., & Carvalho, M. F (2019) Actinobacteria isolated from laminaria ochroleuca: A source of new bioactive compounds. *Frontiers in Microbiology*, *10*(APR), 1–13. https://doi.org/10.3389/fmicb.2019.00683

[23] Wiese, J., Thiel, V., Nagel, K., Staufenberger, T., & Imhoff, J. F. (2009) Diversity of antibiotic active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea. *Mar Biotechnol*, 11: 287–300. https://doi.org/10.1007/s10126-008-9143-4.

[24] Hendrawati, T. Y (2016) Pengolahan Rumput Laut dan Kelayakan Industrinya. UMJ Press.

[25] Hafsan, H., Aziz, I., Sukmawaty, E., S, S., Hasyimuddin, H., Zulkarnain, Z., & Hajrah, H (2019) Antibiotic Activity of Endophytic Bacteria isolated from *Euchema cottonii* of North Galesong Sea, Takalar. *June*. https://doi.org/10.4108/eai.2-5-2019.2284688

[26] Sulistyani, N., & Akbar, A. N (2014) Aktivitas Isolat Actinomycetes dari Rumput Laut (*Eucheuma cottonii*) sebagai Penghasil Antibiotik terhadap *Staphylococcus aureus* dan *Escherichia col*i (Activity of Actinomycetes Isolate from Seeweed (*Eucheuma cottonii*) as Antibiotic Producer against St. *Jurnal Ilmu Kefarmasian Indonesia*, *12*(1), 1–9.

[27] Nur, F., Masri, M., Muthiadin, C., Masriany, M., Latif, U., Rukmana, R., Mustami, M., & Reskianti, R (2019) Isolation and Identification of Endophytic Microbes from *Eucheuma cottonii* of North Galesong Sea, Takalar District. *International Conference on Science and Technology*, *January*. https://doi.org/10.4108/eai.2-5-2019.2284687.

[28] Togatorop, A. P., Dirgayusa, I. G. N. P., & Puspitha, N. L. P. R (2017) Studi Pertumbuhan Rumput Laut Jenis Kotoni (*Eucheuma cottonii*) dengan Menggunakan Metode Kurung Dasar dan Lepas Dasar di Perairan Geger, Bali. *Journal of Marine and Aquatic Sciences*, *3*(1), 47–58. https://doi.org/10.24843/jmas.2017.v3.i01.47-58.

[29] Wiryana, I. W. S. A., Edi, D. G. S., & Kawana, I. M (2018) Potensi Pengembangan Budidaya Rumput Laut *Eucheuma Cottonii* Di Kawasan Perairan Kelurahan Serangan Kota Denpasar Berbasis Sistem Informasi Geografis. *Gema Agro*, *23*(1), 92–103. https://doi.org/10.22225/ga.23.1.663.92-103.

[30] Indraningrat, A. A. G., Micheller, S., Runderkamp, M., Sauerland, I., Becking, L. E., Smidt, H., & Sipkema, D (2019) Cultivation of Sponge-Associated Bacteria from *Agelas sventres* and *Xestospongia muta* Collected from Different Depths. *Marine Drugs*, *17*(10). https://doi.org/10.3390/md17100578.

[31] Smith, A. C., & Hussey, M. A (2005) *Gram stain protocols*. American Society for Microbiology.https://www.asmscience.org/content/education/protocol/protocol.2886.

[32] Reiner, K (2013) *Catalase Test*. https://asm.org/getattachment/72a871fc-ba92-4128-a194-6f1bab5c3ab7/Catalase-Test-Protocol.pdf.

[33] Boontanom, P., & Chantarasiri, A (2020) Short communication: Diversity of culturable

epiphytic bacteria isolated from seagrass (*Halodule uninervis*) in Thailand and their preliminary antibacterial activity. *Biodiversitas*, 21(7):, 2907–2913.

[34] Nedialkova, D., & Naidenova, M (2005) Screening The Antimicrobial Activity Of Actinomycetes Strains Isolated From Antartica. *Journal Of Culture Collections*, *4*, 29–35.

[35] Retnowati, Y (2017) Isolasi dan Uji Aktivitas Antibakteri Actinomycetes dari Rhizosfer Bakau di Hutan Bakau Torosiaje Gorontalo. *Seminar Nasional Pendidikan Biologi Dan Saintek II*, 1–10.

[36] Wilson, W., Purwestri, Y. A., & Sembiring, L (2017) Isolasi, Karakterisasi dan Skrining Antimikrobia Bakteri Endofit Tanaman Purwoceng (*Pimpinella pruatjan* Molk.). *Jurnal Labora Medika*, *1*(1), 01–06.

[37] Wang, F., Li, M., Huang, L., & Zhang, X. H (2021) Cultivation of uncultured marine microorganisms. *Marine Life Science and Technology*, *3*(2), 117–120. https://doi.org/10.1007/s42995-021-00093-z.

[38] Reguera, G (2016) The Great Plate Count Anomaly. In *In the Company of Microbe* (pp. 288–291).

[39] Goecke, F., Labes, A., Wiese, J., & Imhoff, J. F (2010) Review chemical interactions between Marine macroalgae and bacteria. *Marine Ecology Progress Series*, *409*, 267–300. https://doi.org/10.3354/meps08607.

[40] Hettiarachchi, S. A., Lee, S. J., Lee, Y., Kwon, Y. K., De Zoysa, M., Moon, S., Jo, E., Kim, T., Kang, D. H., Heo, S. J., & Oh, C (2017) A rapid and efficient screening method for antibacterial compound-producing bacteria. *Journal of Microbiology and Biotechnology*, *27*(8), 1441–1448. https://doi.org/10.4014/jmb.1703.03012.

[41] Velho-Pereira, S., & Kamat, N. M (2011) *Antimicrobial Screening of Actinobacteria using a Modified Cross-Streak Method*.

[42] Indryani, V., Chiuman, L., Wijaya, L. L., Lister, G., & Grandis, L (2020) Antibacterial Effect of Curcuma zedoaria Extract on *Bacillus cereus* and *Staphylococcus epidermidis*. *7*(March), 6–10.

[43] Awad, H. M., El-Shahed, K. Y. I., Aziz, R., Sarmidi, M. R., & El-Enshasy, H. A. (2012). Antibiotics as Microbial Secondary Metabolites: Production and Application. *Jurnal Teknologi (Sciences & Engineering)*, 59(1), 101-111.

[44] Epand, R. M., Walker, C., Epand, R. F., & Magarvey, N. A (2016) Molecular mechanisms of membrane targeting antibiotics. *Biochimica et Biophysica Acta - Biomembranes*, *1858*(5), 980–987.https://doi.org/10.1016/j.bbamem.2015.10.018.

[45] Ouchari, L., Boukeskasse, A., Bouizgarne, B., & Ouhdouch, Y(2019) Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. *Biology Open*, *8*(2). https://doi.org/10.1242/bio.035410.

[46] Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-vaillant, N., Jacquard, C., Klenk, H., Clément, C., Ouhdouch, Y., & Wezel, P. Van (2016) *Taxonomy , Physiology , and Natural Products of Actinobacteria*. *80*(1), 1–43. https://doi.org/10.1128/MMBR.00019-15.

[47] Li, Q., Chen, X., Jiang, Y., & Jiang, C (2016) Morphological Identification of Actinobacteria. *Actinobacteria - Basics and Biotechnological Applications*, https://doi.org/10.5772/61461.

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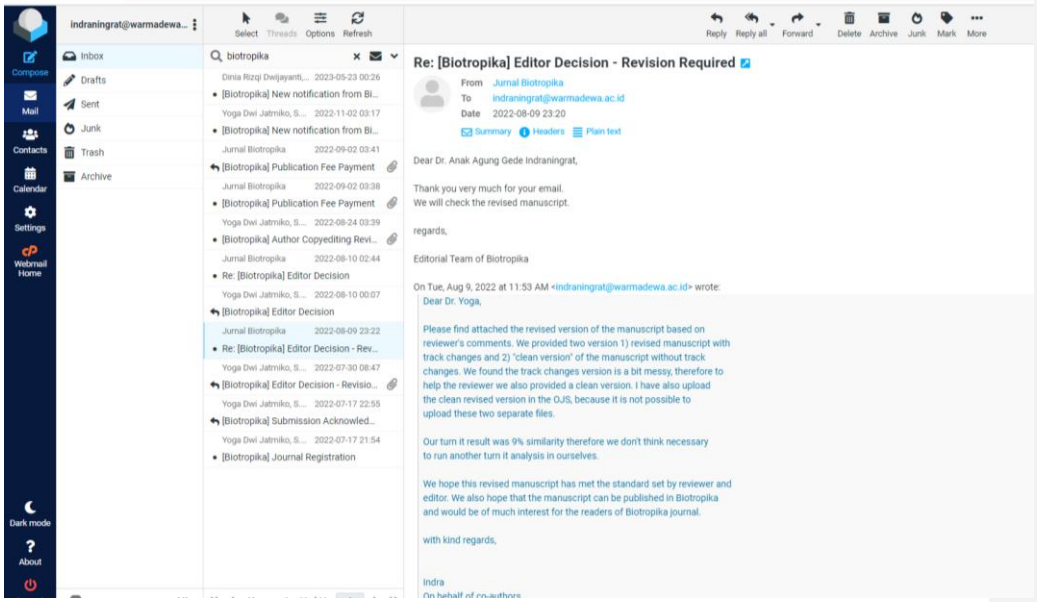

ANTIBACTERIAL ACTIVITY SCREENING OF BACTERIAL ISOLATES ASSOCIATED WITH SEAWEED *Eucheuma cottonii* FROM COASTAL AREA IN BULELENG, BALI

SKRINING AKTIVITAS ANTIBAKTERI ISOLAT BAKTERI YANG BERASOSIASI DENGAN RUMPUT LAUT Eucheuma cottonii DARI AREA PESISIR BULELENG, BALI

Received :

Accepted :

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ABSTRACT

Seaweeds like other marine organisms develop a strong association with prokaryotic communities, especially bacteria. Many of these associated bacteria play an important role in synthesizing secondary metabolites which are beneficial for their host, especially against predation or infection. In Indonesia, Eucheuma cottonii is one of the seaweed species that has been cultivated widely. However, only limited study has been reported on bacterial isolates and their antibacterial activity from E. cottonii. Bacterial isolates from E. cottonii were cultivated in six agar media namely Zobell Marine Agar, Nutrient Agar, Plate Count Agar, ISP-1, ISP-2, and Starch-M protein agar. Cultivation of bacterial isolates yielded 23 isolates with nine bacterial isolates were classified as Gram-positive bacteria and 14 isolates were grouped among Gram-negative bacteria. All the bacterial isolates were screened for their antibacterial activity against six bacterial indicator strains namely Staphylococcus aureus ATCC 25923, Streptococcus mutans FNCC 0405, Escherichia coli ATCC 25922, and Klebsiella pneumoniae ATCC 700603 using perpendicular streak and agar block method. Six out of 23 bacterial isolates displayed antibacterial activity against at least one of the bacterial indicator strains. The bacterial isolate ISP1RL4 showed the highest antibacterial activity with average inhibition of > 20 mm against all bacterial indicators. Overall, our result indicated the potential of bacterial isolates associated with E. cottonii as an antibacterial producer.

Keywords: *antibacterial, Eucheuma cottonii, isolation, seaweed*

ABSTRAK

Rumput laut seperti organisme laut lainnya membangun asosiasi yang kuat dengan komunitas prokariotik khususnya bakteri. Banyaknya bakteri yang berasosiasi ini berperan penting dalam mensintesis senyawa metobolit sekunder yang bermanfaat bagi inangnya, khususnya terhadap predasi atau infeksi. Di Indonesia, Eucheuma cottonii ialah salah satu spesies rumput laut yang telah dibudidayakan secara luas. Namun, sejauh ini masih terbatas penelitian yang melaporkan isolat bakteri dan aktivitas antibakterinya dari E. cottonii. Bakteri yang berasosiasi dengan E. cottonii dikultivasi pada enam media agar yaitu Zobell Marine Agar, Nutrient Agar, Plate Count Agar, ISP-1, ISP-2, dan Strach-M Protein agar. Hasil isolasi bakteri mendapatkan 23 isolat dengan sembilan isolat bakteri tergolong bakteri Gram positif dan 14 isolat tergolong bakteri Gram negatif. Seluruh isolat bakteri diskrining aktivitas antibakterinya terhadap enam jenis bakteri indicator yaitu Staphylococcus aureus ATCC 25923, Streptococcus mutans FNCC 0405, Escherichia coli ATCC 25922 dan Klebsiella pneumoniae ATCC 700603 menggunakan metode goresan tegak lurus dan blok agar. Enam dari 23 isolat bakteri menunjukkan aktivitas antibakteri terhadap setidaknya satu jenis bakteri indikator. Isolat bakteri ISP1RL4 menampilkan aktivitas antibakteri tertinggi dengan rata-rata zona hambat > 20 mm terhadap seluruh bakteri indikator. Secara keseluruhan, hasil kami menunjukkan potensi isolat bakteri yang berasosiasi dengan E. cottonii sebagai penghasil antibakteri.

How to cite:

INTRODUCTION

Infection by pathogenic bacteria is still the leading cause of death globally, especially in developing countries [1]. The incidence of infection diseases is estimated in the ranged between 3.5% and 12% in high income countries, whereas it varies between 5.7% and 19.1% in middle and low-income countries [2]. The high incidence of infectious diseases worldwide is also influenced by the increasing rate of antimicrobial resistance [2,3]. The existence of antibiotic resistance in some pathogenic bacteria causes the use of antibiotics or other antimicrobial drugs to be ineffective and infections to become increasingly difficult or impossible to treat [4]. Therefore, the search for new antibiotic compounds originating from rare and natural sources will yield useful clues in the identification of new drugs against the increasing resistance of pathogenic bacteria [5].

So far, efforts to search for new antibacterial compounds have been focused on terrestrial ecosystems. However, the discovery rate of antibacterial compounds from terrestrial sources is often limited by the discovery of existing compounds (dereplication) [6]. Therefore, current studies have been focused to search for novel antibacterial compounds from marine ecosystem since they are rather unexplored and offer a source of unique secondary metabolites [7].

Among marine organisms, seaweeds are one of the

producers for secondary metabolites including antibacterial compounds such as halogenated compounds, sterols, heterocyclics, and phenolic compounds that showed antibacterial effects against Gram-positive and negative bacteria [8]. In addition, seaweed naturally builds beneficial associations especially with bacteria by providing a suitable substrate for bacterial colonization and also secretes various organic substances that function as nutrients for bacterial propagation [9]. On the other hand, bacteria associated with seaweed actively synthesize various secondary metabolites to protect the host from infection or predation [10].

Currently, research has been focused to isolate seaweeds-associated bacteria to provide sustainable source of secondary metabolites for research and development. Seaweeds are known to host various species of Actinobacteria, both epiphytic and endophytic. About 80% of antibiotics are mainly produced by Actinobacteria, especially the genera Streptomyces and Micromonospora [11]. Significant efforts have been focused on the isolation of novel marine actinobacteria to develop drug discovery especially antibiotics [12]. Several studies have shown that actinobacteria isolated from seaweed are capable of producing bioactive compounds, including antibiotics [13, 14] and showed antibacterial activity against several pathogenic bacteria, such as *Proteus* sp., *Enterobacter* sp. [15], *Vibrio alginolyticus* [12], and *S. aureus* [16]. Apart from actinobacterial isolates, other bacteria that associated with seaweed has also been reported to show antibacterial activity such as *Pseudomonas* sp, *Stenotrophomonas* sp, *Vibrio* sp, *Altomonas* sp, *Shewanella* sp, and *Bacillus* sp. [17].

One of the most common types of seaweed found in Indonesian seawater is *Eucheuma cottonii*, which is cultivated for the food and non-food product industry [18]. The association of bacteria with seaweed *E. cottonii* has been shown to provide antibacterial activity [19, 20]. A study has reported that two bacterial isolates were isolated from *E. cottonii* seaweed in the North Galesong Sea, Takalar, South Sulawesi, namely *Aeromonas* sp. and *Klebsiella* sp. [21]. A follow up study reported that the isolate *Aeromonas* sp. was the only bacterial species with antibacterial activity against *S. aureus* and *E. coli* [19]. Another study on the cultivation of bacterial isolates from seaweed *E. cottonii* from Bangsal beach, Lombok, West Nusa Tenggara reported two Actinomycetes isolate namely RL 6 and RL 12 have inhibited the growth of *S. aureus* and *E. coli* with zone of inhibition of >30 mm [20].

Studies on the isolation, characterization, and screening of the antibacterial activity of the bacterial community associated with *E. cottonii* seaweed from the coastal waters of Bali are rather limited. To date, studies on *E. cottonii* seaweed in coastal waters in Bali were more focused to analyze growth rates and potential for aquaculture development [22, 23]. Therefore, this study was aimed to identify morphology of bacterial isolates associated with seaweed *E. cottonii* and to screen for antibacterial activities of these isolates against Gram-positive and Gram-negative bacterial indicator strains. It is expected that the outcome of this research will lead for a bacterial isolate with strong antibacterial activity.

METHODS

Sampling of *E. cottonii*. This research was conducted from November 2021 to March 2022 at the Laboratory Agriculture, Faculty of Agriculture, Warmadewa University. Samples of *E. cottonii* were taken from the beach in Patas Singaraja Village, Gerokgak District, Buleleng Regency, Bali Province (8°11'09.6"S, 114°48'48.6"E). Approximately 100 g of *E. cottonii* was taken aseptically using sterile gloves and a knife. The obtained samples were stored in a sterile Falcon tube and closed tightly. In addition, 1 L of seawater was taken from the sampling location and stored in a sterile glass bottle (Durant). Samples of *E. cottonii* and seawater were stored in a cool box that already contained an ice pack. Subsequently, samples were transported to the laboratory and stored at 4 °C until further testing.

Cultivation conditions. Samples of *E. cottonii* seaweed were washed with sterile artificial seawater three times, weighed 10 g, and cut into pieces to ensure that all thallus parts, including the interior and exterior, were evenly mixed. The sample was homogenized by pounding it under sterile conditions with mortar and pastel and then adding 25 mL of sterile artificial seawater. A total of 1 mL of *E. cottonii* seaweed suspension was diluted in stages in a test tube containing 9 mL of sterile artificial seawater (10^{-1} to 10^{-6}). Each dilution of 10^{-3} to 10^{-5} was taken as much as 200 µL to be planted on a predetermined cultivation media and spread using a sterile cotton swab [24]. The following six cultivation media were used which three of them were aimed for non-actinobacterial species namely Zobell Marine Agar (Himedia), Nutrient Agar (Himedia), and Plate Count Agar (Oxoid). In addition, three other media were aimed specifically for actinobacterial isolates namely ISP-1 (5.0 g/L peptone, 3.0 g/L yeast extract), ISP-2 (4.0 g/L yeast extract, 10 g/L malt extract, 4 g/L dextrose) and Starch-M protein agar (Himedia). Four media namely NA, PCA, ISP-1, and ISP-2 were dissolved in artificial seawater (33 g/L). All media were 20 g/L of bacto agar (Himedia) to produce solid media. After autoclaving, ISP-1, ISP-2, and Starch M-protein agar media were supplemented with Nystatin (800 µL) and nalidixic acid (200 µL). Petri dishes containing agar media and samples were then wrapped in parafilm and incubated upside down in an incubator at 28 °C. Periodic

observations were made every 3 days for a maximum of two weeks to count the number of colonies that appeared on each agar media. Individual bacteria colonies with different morphologies that grow on media were picked and characterized according to the colony morphology code (CMC) criteria [25] (Figure 1). Non-Actinobacterial isolates were purified on Zobell marine agar media, while Actinobacteria candidate isolates were purified on ISP-2 agar. The selected colonies with different morphology were colorized based on Gram staining procedure [26] and catalase test [27]. Subsequently, cell morphology was observed under light microscope (Leica DM750).

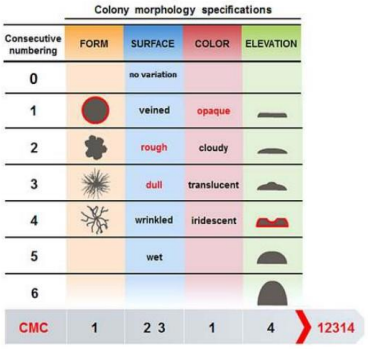

Figure 1. Coding of bacterial colony morphology based on the CMC method [25].

Screening of antibacterial activity. Antibacterial activity screening was carried out using two methods, namely perpendicular streak [27] for non-actinobacterial isolates and agar block method [30, 31, 32] for actinobacterial candidates. The reason to use two different screening methods was based on characteristics of actinobacterial isolates that are generally grip on agar media and they grow slower compared to that of non-actinobacterial isolates. Briefly, bacterial isolates were streaked on LB agar perpendicularly with distance of 8 cm and subsequently the agar plate were incubated for 48 hours at 28 ^{o}C until colonies were formed on agar. Furthermore, four bacterial indicator strains namely *Staphylococcus aureus* ATCC 25923, *Streptococcus mutans* FNCC 0405 *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 700603 were streaked horizontally from the tested isolates with distance of 3 cm (Figure 2). Subsequently, agar plates were incubated for 24 hours at 37 ^{o}C and the length of the inhibition zone (in mm) formed on each test bacteria was measured [28, 29]. The zone of inhibition was calculated based on the distance of bacterial growth by bacterial isolates to each of the tested bacteria.

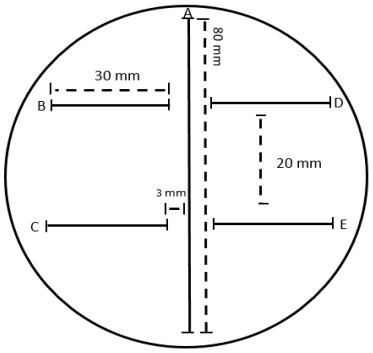

Figure 2. Streaking pattern for screening using the perpendicular streak method [27]. A. Isolates of test bacteria, B. *Staphylococcus aureus* ATCC 25923, C. *Streptococcus mutans* FNCC 0405, D. *Escherichia coli* ATCC 25922, E. *Klebsiella pneumoniae* ATCC 700603.

Agar block method started by refreshing the four bacterial indicator strains in LB broth. Furthermore, 200 µL of suspension for each test bacterium with an optical density (OD) of 0,5 was spread with a sterile cotton swab on LB agar. Subsequently, the actinobacteria isolates that had been purified on ISP-2 agar media with a minimum age of 10 days were cut into blocks of 1 x 1 cm using a sterile scalpel. Block pieces for actinobacterial isolates were then placed in each petri dish containing the test bacteria and incubated at 37 ^{o}C for 48 hours. The antibacterial activity against bacterial indicator strains was observed by the formation of a clear zone around the agar block [31]. The diameter of clear zone was measured using a digital caliper.

RESULTS AND DISCUSSION

Morphological characteristics of bacterial isolates. A total of 23 bacterial isolates were isolated from *E. cottonii* which further can be divided into 11 isolates from general media and 12 isolates from specific media targeting for Actinobacteria (Table 1A and Table 1B). Majority of isolates obtained from general media had an irregular shape, a dull surface, an opaque color, and a flat elevation. Meanwhile, bacterial isolates from specific media have more diverse appearances such as irregular shapes, and circular. Moreover, their surface tended to be rough and dull, color was mostly opaque and elevations that vary from flat, raised, umbonate, to crateriform. Cell wall composition of these isolates were dominantly Gram-negative with 14 bacterial isolates (70%) and the remaining were Gram-positive bacteria with 9 bacterial isolates (30%). Furthermore, 20 bacterial isolates (87%) have positive catalase activity and three

bacterial isolates (13%) have negative catalase.

Twenty-three isolates of bacteria have individual characteristics so they are considered to come from different types of bacteria. Actinobacterial isolates were in general characterized by Gram-positive, coccus and rod-shaped cells. Apart from the obtained number of colonies that vary, it cannot be denied that not all bacteria associated with *E. cottonii* can be isolated. Naturally, only a small fractions of bacteria associated with a organism can be cultured under the standard culture system carried out for the laboratory scale. This is influenced by factors of inadequate growth conditions, low growth rates, the need for metabolites produced by other bacteria, and the presence of inactive cells [33] so it is estimated that only about 1 in 100 microbes can be cultured, which refers to phenomenon of "the great plate count anomaly" [34]. To date, most of the available information on bacterial and seaweed interactions has been obtained from culture-based studies, and it is estimated that only 1 to 10% of associated bacteria can be cultured [35].

Evaluation of the antibacterial activity of bacterial isolates associated with *E. cottonii*. Antibacterial activity screening of isolates associated with *E. cottonii* became the main focus of this study to obtain potential isolates that could produce antibacterial compounds. Screening carried out to find new antibacterial compounds must be simple, fast, repeatable, and inexpensive [36]. The antibacterial activity screening method used consisted of two methods, namely the perpendicular streak method and the agar block method. These methods were chosen because these procedure are easy, fast, inexpensive, and can provide rapid screening results for antagonistic tests on bacteria [37].

All bacterial isolates on general media could not provide antibacterial activity against the four test bacteria which were indicated by the absence of a clear distance formed in the streaked area of the test bacteria (Table 2). Meanwhile, six bacterial isolates that were obtained from specific media that could provide antibacterial activity against the test bacteria, namely isolates with codes ISP1RL2, ISP1RL3, ISP1RL4, ISP1RL5, ISP1RL6, and SMPRL2 (Figure 3). Zone of inhibition ISP1RL2, ISP1RL3, ISP1RL4, ISP1RL5, ISP1RL6, and SMPRL2 on agar block methods could be classified into four categories, namely weak (<5 mm), moderate (5-10 mm), strong (10-20 mm), and very strong (>20 mm) [38]. These six isolates were able to inhibit at least one isolate of the test bacteria and showed very strong antibacterial activity with a mean inhibition zone of more than 20 mm, except for isolates ISP1RL5 and ISP1RL6 which had a weak inhibition for *K. pneumoniae*. The highest inhibition zone for each test bacteria can be seen in Figure 4.

Differences in the zone of inhibition produced by actinobacterial isolates may depend on secondary metabolites produced by each bacterial isolate. Secondary metabolite compounds are very sensitive to changes in environmental and cultural conditions [39]. Therefore, the *in vitro* production of most antibiotics depends on the composition of the medium in which the production microorganisms are grown [39]. Based on this, it can be assumed that the nutritional composition of LB agar media used in the antibacterial activity test cannot accommodate optimal bacterial growth, so that the bacteria cannot produce their secondary metabolites causing no inhibition zones to be formed.

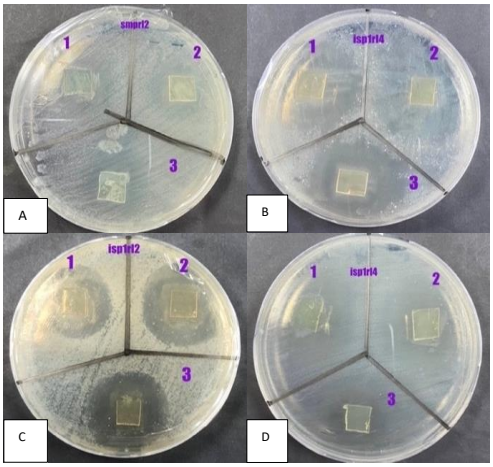

Figure 4. Zone of inhibition of bacterial isolates from *E. cottonii* against test bacteria. A. ISP1RL2 isolate had the highest zone of inhibition against *S. aureus* test bacteria at 29,4 mm; B. ISP1RL4 isolate had the highest inhibition zone against the test bacteria *S. mutans* at 35,4 mm; C. ISP1RL2 isolate had the highest zone of inhibition against *E. coli* test bacteria at 31,6 mm; D. ISP1RL4 isolate had the highest zone of inhibition against the test bacteria *K. pneumoniae* at 31,1 mm.
Description: 1 = the first replicate ; 2 = second replicate; 3 = third replicate

Table 1. Morphology of bacterial isolates according to CMC, Gram stain, and catalase

A. Morphology of Bacterial Isolates from *E. cottonii* on General Media

Isolate code	Morphology of colony characteristic				Type of Gram	Cell Shape	Catalase
	Form	Surface	Colour	Elevation			
ZRL1	2 (Irregular)	3 (Dull)	1 (Opaque)	1 (Flat)	-	Coccus	+
ZRL2	1 (Circular)	3 (Dull)	3 (Translucent)	2 (Raised)	-	Coccus	+
NRL1	1 (Circular)	3 (Dull)	1 (Opaque)	1 (Flat)	-	Coccus	-
NRL2	1 (Circular)	3 (Dull)	1 (Opaque)	1 (Flat)	+	Coccus	+
NRL3	1 (Circular)	1 (Veined)	1 (Opaque)	1 (Flat)	+	Coccus	+
NRL4	2 (Irregular)	1 (Veined)	1 (Opaque)	1 (Flat)	-	Coccus	-
PCARL1	2 (Irregular)	3 (Dull)	1 (Opaque)	1 (Flat)	+	Coccus	+
PCARL2	2 (Irregular)	3 (Dull)	3 (Translucent)	1 (Flat)	-	Bacilli	+
PCARL3	2 (Irregular)	24 (Rough and wrinkled)	3 (Translucent)	1 (Flat)	-	Bacilli	+
PCARL4	1 (Circular)	3 (Dull)	1 (Opaque)	1 (Flat)	+	Bacilli	+
PCARL5	2 (Irregular)	23 (Rough and dull)	1 (Opaque)	1 (Flat)	-	Coccus	+

B. Morphology of Bacterial Isolates from *E. cottonii* on Specific Media

Isolate code	Morphology of colony characteristic				Type of Gram	Cell Shape	Catalase
	Form	Surface	Colour	Elevation			
ISP1RL1	2 (Irregular)	34 (Dull and wrinkled)	1 (Opaque)	4 (Crateriform)	+	Bacillus	+
ISP1RL2	1 (Circular)	2 (Rough)	1 (Opaque)	2 (Raised)	-	Bacillus	-
ISP1RL3	1 (Circular)	3 (Dull)	3 (Translucent)	1 (Flat)	-	Bacillus	-
ISP1RL4	1 (Circular)	34 (Dull and wrinkled)	4 (Yellow)	2 (Raised)	+	Coccus	+
ISP1RL5	2 (Irregular)	3 (Dull)	1 (Opaque)	1 (Flat)	-	Bacillus	-
ISP1RL6	2 (Irregular)	23 (Rough and dull)	1 (Opaque)	1 (Flat)	-	Bacillus	-
ISP2RL1	2 (Irregular)	23 (Rough and dull)	4 (Yellow)	3 (Umbonate0	-	Bacillus	-
ISP2RL2	1 (Circular)	3 (Dull)	1 (Opaque)	2 (Raised)	-	Bacillus	-
ISP2RL3	3 (Filamentous)	23 (Rough and dull)	1 (Opaque)	1 (Flat)	+	Bacillus	+
SMPRL1	2 (Irregular)	34 (Dull and wrinkled)	1 (Opaque)	4 (Crateriform)	+	Bacillus	+
SMPRL2	2 (Irregular)	3 (Dull)	4 (Yellow)	2 (Raised)	+	Bacillus	+
SMPRL3	2 (Irregular)	23 (Rough and dull)	1 (Opaque)	2 (Raised)	-	Bacillus	-

Description: ISP1RL: ISP-1 Agar Media, ISP2: ISP-2 Agar Media, SMPRL: Starch-M Protein Agar Media, +: Positive, -: Negative.

Table 2. Antibacterial Activity of Bacteria Isolated from *E. cottonii*

Zone of Inhibition of Bacterial Isolates from General Media					
No	Sample code	Zone of inhibition (mm)			
		Staphylococcus aureus ATCC 25923	*Streptococcus mutans* FNCC 0405	*Escherichia coli* ATCC 25922	*Klebsiella pneumoniae* ATCC 700603
1	ZRL1	-	-	-	-
2	ZRL2	-	-	-	-
3	NRL1	-	-	-	-
4	NRL2	-	-	-	-
5	NRL3	-	-	-	-
6	NRL4	-	-	-	-
7	PCARL1	-	-	-	-
8	PCARL2	-	-	-	-
9	PCARL3	-	-	-	-
10	PCARL4	-	-	-	-
11	PCARL5	-	-	-	-

Zone of Inhibition of Bacterial Isolates from Specific Media

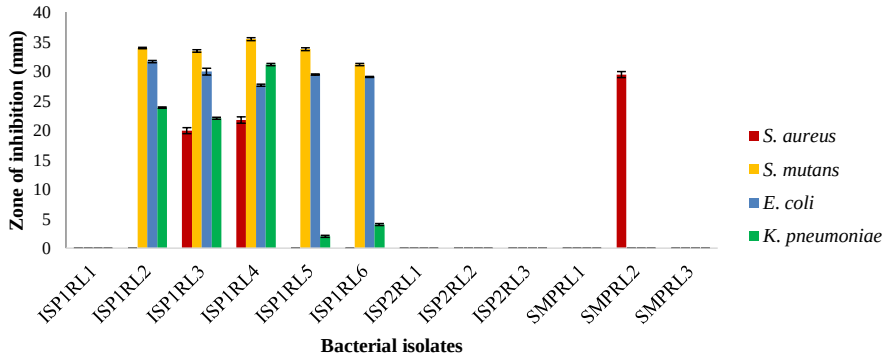

Figure 3. Zone of inhibition of bacterial isolates from specific media targeting for Actinobacteria against the four bacterial indicator strains based on agar block method.

The inhibition zones formed on each test bacteria varied greatly with the range of inhibition zones ranging from 20–30 mm, 31–34 mm, 27–32 mm 2–32 mm, respectively for *S. aureus* ATCC 25923, *S. mutans* FNCC 0405, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 700603 (Figure 3). In general, these active isolates were more active against Gram-positive bacteria *S. aureus* and *S. mutans* than the Gram-negative bacteria *E. coli* and *K. pneumoniae*. Such discrepancies of antibacterial activity could happen because Gram-negative bacteria have a better defense system against antibacterial compounds than Gram-positive bacteria, which is influenced by differences in the cell wall components between the two group of bacteria [40]. In addition, Gram-negative bacteria also have an outer membrane, that composed mainly of lipopolysaccharide which covers 90% of the cell surface and functions as an additional protection system, so that Gram-negative bacteria are more resistant against antibacterial compounds [40, 41].

Based on the results of macroscopic and microscopic identification, and the presence of antibacterial activity, two candidate isolates of bacteria were chosen, namely isolates ISP1RL4 and SMPRL2 (Figure 5) with characteristics resembles Actinobacteria. These two isolates have Gram-positive, coccus and rod-shaped cells, have antibacterial activity commonly found in the Actinobacteria group [42], aerial mycelium with gray-yellow pigmentation on ISP1RL4, and the presence of pale-yellow aerial spores on SMPRL2, and slow growth [43]. Of the two isolates, ISP1RL4 was considered as the best actinobacterial candidate with the highest range of inhibition for all test bacteria.

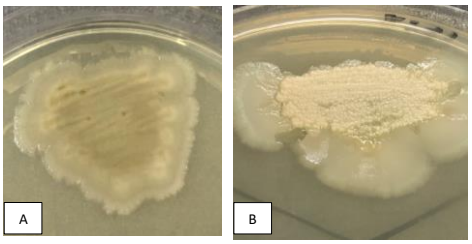

Figure 5. Pure isolates of Actinobacteria candidates (a) ISP1RL4 isolate; (b) SMPRL2 isolate.

The antibacterial screening of the six bacterial isolates from *E. cottonii*: ISP1RL2, ISP1RL3, ISP1RL4, ISP1RL5, ISP1RL6, and SMPRL2 show specificity of each isolate against each type of Gram-positive or Gram-negative bacteria. Further research needs to be focused to explore bioactivity of these isolates against multidrug resistant bacteria and also against other microbes such as parasitic fungi dan parasites.

CONCLUSION

In conclusion, a total of 23 bacterial isolates were isolated from *E. cottonii* seaweed. Six bacterial isolates namely ISP1RL2, ISP1RL3, ISP1RL4, ISP1RL5, ISP1RL6, and SMPRL2 displayed antibacterial activity against at least one of bacterial indicator strains. Among the six isolates, ISP1RL4 showed the highest antibacterial activities. Further research will be focused to sequence the six isolates by amplifying 16S rRNA gene fragment.

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REFERENCES

[1] Sarmah, P., Dan, M. M., Adapa, D., & TK, S. (2018). A review on common pathogenic

microorganisms and their impact on human health. *Electronic Journal of Biology*, 14(1), 50–58.

[2] Khan, A. H., Baig, F. K., & Mehbob, R. (2018). Nosocomial infections: Epidemiology, prevention, control, and surveillance. *Asian Pacific Journal of Tropical Biomedicine*, 7(5), 478-482. https://doi.org/10.1016/j.apjtb.2017.01.019

[3] Nagel, T. E., Chan, B. K., De Vos, D., El-Shibiny, A., Kang'ethe, E. K., Makumi, A., & Pirnay, J-P. (2016) The Developing World Urgently Needs Phages to Combat Pathogenic Bacteria. *Front. Microbiol.* 7:882. https://doi.org/10.3389/fmicb.2016.00882.

[4] WHO (2020) *Antimicrobial resistance*. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

[5] Ali, S. M., Siddiqui, R., & Khan, N. A. (2018) Antimicrobial discovery from natural and unusual sources. *Journal of Pharmacy and Pharmacology*, 70(10):, 1287–1300.

[6] Cox, G., Sieron, A., King, A. M., De Pascale, G., Pawlowski, A. C., Koteva, K., & Wright, G. D. (2017). A Common Platform for Antibiotic Dereplication and Adjuvant Discovery. *Cell Chemical Biology*, *24*(1), 98–109. https://doi.org/10.1016/j.chembiol.2016.11.011.

[7] Hai, Y., Wei, M. Y., Wang, C. Y., Gu, Y. C., & Shao, C. L (2021) The intriguing chemistry and biology of sulfur-containing natural products from marine microorganisms (1987–2020). *Marine Life Science and Technology*, *3*(4), 488–518. https://doi.org/10.1007/s42995-021-00101-2

[8] Salehi, B., Sharifi-Rad, J., Seca, A. M. L., Pinto, D. C. G. A., Michalak, I., Trincone, A., Mishra, A. P., Nigam, M., Zam, W., & Martins, N (2019) Current trends on seaweeds: Looking at chemical composition, phytopharmacology, and cosmetic applications. *Molecules*, 24(22).

[9] Singh, R. P., & Reddy, C. R. K (2014) Seaweed-microbial interactions: Key functions of seaweed-associated bacteria. *FEMS Microbiology Ecology*, 88(2):, 213–230.

[10] Egan, S., Harder, T., Burke, C., Steinberg, P., Kjelleberg, S., & Thomas, T (2013) The seaweed holobiont: Understanding seaweed-bacteria interactions. *FEMS Microbiology Reviews*, 37(3):, 462–476.

[11] Anandan, R., Dharumadurai, D., & Manogaran, G. P. (2016). An Introduction to Actinobacteria. *Actinobacteria - Basics and Biotechnological Apllications*. IntechOpen.

[12] Ulfah, M., Kasanah, N., & Handayani, N. S. N (2018) Bioactivity and genetic screening of marine actinobacteria associated with red algae *Gelidiella acerosa*. *Indonesian Journal of Biotechnology*, *22*(1), 13–21. https://doi.org/10.22146/ijbiotech.25920

[13] Braña, A. F., Fiedler, H. P., Nava, H., González, V., Sarmiento-Vizcaíno, A., Molina, A., Acuña, J. L., García, L. A., & Blanco, G (2015) Two Streptomyces Species Producing Antibiotic, Antitumor, and Anti-Inflammatory Compounds Are Widespread Among Intertidal Macroalgae and Deep-Sea Coral Reef Invertebrates from the Central Cantabrian Sea. *Microbial Ecology*, *69*(3), 512–524. https://doi.org/10.1007/s00248-014-0508-0

[14] Uzair, B., Menaa, F., Khan, B. A., Mohammad, F. V., Ahmad, V. U., Djeribi, R., & Menaa, B (2018) Isolation, purification, structural elucidation and antimicrobial activities of kocumarin, a novel antibiotic isolated from actinobacterium *Kocuria marin*a CMG S2 associated with the brown seaweed *Pelvetia canaliculata. Microbiological Research*, *206*, 186–197.https://doi.org/10.1016/j.micres.2017.10.007

[15] Negara, B. F. S. P., Riyanti, ., Marhaeni, B., & Kusuma, A. B (2016) Antibacterial activity

of Actinomycetes symbiont with seaweeds: a prosperous agent of animal antibacterial. *Aceh Journal of Animal Science*, *1*(2), 45–49. https://doi.org/10.13170/ajas.1.2.4475

[16] Girão, M., Ribeiro, I., Ribeiro, T., Azevedo, I. C., Pereira, F., Urbatzka, R., Leão, P. N., & Carvalho, M. F (2019) Actinobacteria isolated from laminaria ochroleuca: A source of new bioactive compounds. *Frontiers in Microbiology*, *10*(APR), 1–13. https://doi.org/10.3389/fmicb.2019.00683

[17] Wiese, J., Thiel, V., Nagel, K., Staufenberger, T., & Imhoff, J. F. (2009) Diversity of antibiotic active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea. *Mar Biotechnol,* 11: 287–300. https://doi.org/10.1007/s10126-008-9143-4.

[18] Hendrawati, T. Y (2016) Pengolahan Rumput Laut dan Kelayakan Industrinya. UMJ Press.

[19] Hafsan, H., Aziz, I., Sukmawaty, E., S, S., Hasyimuddin, H., Zulkarnain, Z., & Hajrah, H (2019) Antibiotic Activity of Endophytic Bacteria isolated from *Euchema cottonii* of North Galesong Sea, Takalar. *June*. https://doi.org/10.4108/eai.2-5-2019.2284688

[20] Sulistyani, N., & Akbar, A. N (2014) Aktivitas Isolat Actinomycetes dari Rumput Laut (*Eucheuma cottonii*) sebagai Penghasil Antibiotik terhadap *Staphylococcus aureus* dan *Escherichia col*i (Activity of Actinomycetes Isolate from Seeweed (*Eucheuma cottonii*) as Antibiotic Producer against St. *Jurnal Ilmu Kefarmasian Indonesia*, *12*(1), 1–9.

[21] Nur, F., Masri, M., Muthiadin, C., Masriany, M., Latif, U., Rukmana, R., Mustami, M., & Reskianti, R (2019) Isolation and Identification of Endophytic Microbes from *Eucheuma cottonii* of North Galesong Sea, Takalar District. *International Conference on Science and Technology*, *January*. https://doi.org/10.4108/eai.2-5-2019.2284687.

[22] Togatorop, A. P., Dirgayusa, I. G. N. P., & Puspitha, N. L. P. R (2017) Studi Pertumbuhan Rumput Laut Jenis Kotoni (*Eucheuma cottonii*) dengan Menggunakan Metode Kurung Dasar dan Lepas Dasar di Perairan Geger, Bali. *Journal of Marine and Aquatic Sciences*, *3*(1), 47–58. https://doi.org/10.24843/jmas.2017.v3.i01.47-58.

[23] Wiryana, I. W. S. A., Edi, D. G. S., & Kawana, I. M (2018) Potensi Pengembangan Budidaya Rumput Laut *Eucheuma Cottonii* Di Kawasan Perairan Kelurahan Serangan Kota Denpasar Berbasis Sistem Informasi Geografis. *Gema Agro*, *23*(1), 92–103. https://doi.org/10.22225/ga.23.1.663.92-103.

[24] Öner, O., Ekiz, G., Hames, E. E., Demir, V., Gübe1, Ö., Özkaya, F. C., Yokeş, M. B., Uzel, A., & Bedir, E (2014) Cultivable Sponge-Associated Actinobacteria from Coastal Area of Eastern Mediterranean Sea. *Advances in Microbiology*, *4*, 306-316. http://dx.doi.org/10.4236/aim.2014.46037

[25] Indraningrat, A. A. G., Micheller, S., Runderkamp, M., Sauerland, I., Becking, L. E., Smidt, H., & Sipkema, D (2019) Cultivation of Sponge-Associated Bacteria from *Agelas sventres* and *Xestospongia muta* Collected from Different Depths. *Marine Drugs*, *17*(10). https://doi.org/10.3390/md17100578.

[26] Smith, A. C., & Hussey, M. A (2005) *Gram stain protocols*. American Society for Microbiology.https://www.asmscience.org/content/education/protocol/protocol.2886.

[27] Reiner, K (2013) *Catalase Test*. https://asm.org/getattachment/72a871fc-ba92-4128-a194-6f1bab5c3ab7/Catalase-Test-Protocol.pdf.

[28] Boontanom, P., & Chantarasiri, A (2020) Short communication: Diversity of culturable epiphytic bacteria isolated from seagrass (*Halodule uninervis*) in Thailand and their preliminary antibacterial activity. *Biodiversitas*, 21(7), 2907–2913.

[29] Indraningrat, A. A. G., Wijaya, M. D., Suryandhita, P. A., Siskayani, A. S., Janurianti, N. M. D (2021) Antibacterial Screening of Bacterial Isolates Associated with Mangrove Soil from the Ngurah Rai Mangrove Forest Bali. *Biology, Medicine, & Natural Product Chemistry*, 10(2), 129-133.

[30] Nedialkova, D., & Naidenova, M (2005) Screening The Antimicrobial Activity Of Actinomycetes Strains Isolated From Antartica. *Journal Of Culture Collections*, *4*, 29–35.

[31] Retnowati, Y (2017) Isolasi dan Uji Aktivitas Antibakteri Actinomycetes dari Rhizosfer Bakau di Hutan Bakau Torosiaje Gorontalo. *Seminar Nasional Pendidikan Biologi Dan Saintek II*, 1–10.

[32] Wilson, W., Purwestri, Y. A., & Sembiring, L (2017) Isolasi, Karakterisasi dan Skrining Antimikrobia Bakteri Endofit Tanaman Purwoceng (*Pimpinella pruatjan* Molk.). *Jurnal Labora Medika*, *1*(1), 01–06.

[33] Wang, F., Li, M., Huang, L., & Zhang, X. H (2021) Cultivation of uncultured marine microorganisms. *Marine Life Science and Technology*, *3*(2), 117–120. https://doi.org/10.1007/s42995-021-00093-z.

[34] Reguera, G (2016) The Great Plate Count Anomaly. In *In the Company of Microbe* (pp. 288–291).

[35] Goecke, F., Labes, A., Wiese, J., & Imhoff, J. F (2010) Review chemical interactions between Marine macroalgae and bacteria. *Marine Ecology Progress Series*, *409*, 267–300. https://doi.org/10.3354/meps08607.

[36] Hettiarachchi, S. A., Lee, S. J., Lee, Y., Kwon, Y. K., De Zoysa, M., Moon, S., Jo, E., Kim, T., Kang, D. H., Heo, S. J., & Oh, C (2017) A rapid and efficient screening method for antibacterial compound-producing bacteria. *Journal of Microbiology and Biotechnology*, *27*(8), 1441–1448. https://doi.org/10.4014/jmb.1703.03012.

[37] Armengol, E. S., Harmanci, M., & Laffleur, F (2021) Current strategies to determine antifungal and antimicrobial activity of natural compounds. *Microbiological Research*, *252*. https://doi.org/10.1016/j.micres.2021.126867

[38] Indryani, V., Chiuman, L., Wijaya, L. L., Lister, G., & Grandis, L (2020) Antibacterial Effect of Curcuma zedoaria Extract on *Bacillus cereus* and *Staphylococcus epidermidis*. *7*(March), 6–10.

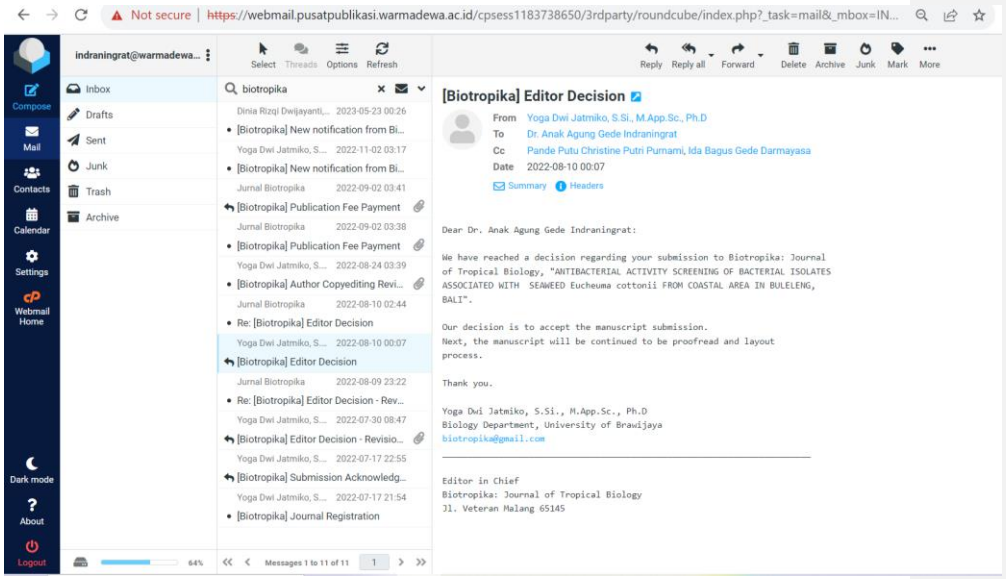
[39] Awad, H. M., El-Shahed, K. Y. I., Aziz, R., Sarmidi, M. R., & El-Enshasy, H. A. (2012). Antibiotics as Microbial Secondary Metabolites: Production and Application. *Jurnal Teknologi (Sciences & Engineering)*, 59(1), 101-111.

[40] Epand, R. M., Walker, C., Epand, R. F., & Magarvey, N. A (2016) Molecular mechanisms of membrane targeting antibiotics. *Biochimica et Biophysica Acta - Biomembranes*, *1858*(5), 980–987.https://doi.org/10.1016/j.bbamem.2015.10.018.

[41] Ouchari, L., Boukeskasse, A., Bouizgarne, B., & Ouhdouch, Y(2019) Antimicrobial potential of actinomycetes isolated from the unexplored hot Merzouga desert and their taxonomic diversity. *Biology Open*, *8*(2). https://doi.org/10.1242/bio.035410.

[42] Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-vaillant, N., Jacquard, C., Klenk, H., Clément, C., Ouhdouch, Y., & Wezel, P. Van (2016) *Taxonomy , Physiology , and Natural Products of Actinobacteria*. *80*(1), 1–43. https://doi.org/10.1128/MMBR.00019-15.

[43] Li, Q., Chen, X., Jiang, Y., & Jiang, C (2016) Morphological Identification of Actinobacteria. *Actinobacteria - Basics and Biotechnological Applications*, https://doi.org/10.5772/61461.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[Biotropika] Editor Decision
From Yoga Dwi Jatmiko, S.Si., M.App.Sc., Ph.D
To Dr. Anak Agung Gede Indraningrat
Cc Pande Putu Christine Putri Purnami, Ida Bagus Gede Darmayasa
Date 2022-08-10 00:07
Summary Headers
Dear Dr. Anak Agung Gede Indraningrat:
We have reached a decision regarding your submission to Biotropika: Journal of Tropical Biology, "ANTIBACTERIAL ACTIVITY SCREENING OF BACTERIAL ISOLATES ASSOCIATED WITH SEAWEED Eucheuma cottonii FROM COASTAL AREA IN BULELENG, BALI".
Our decision is to accept the manuscript submission.
Next, the manuscript will be continued to be proofread and layout process.
Thank you.
Yoga Dwi Jatmiko, S.Si., M.App.Sc., Ph.D
Biology Department, University of Brawijaya
biotropika@gmail.com
Editor in Chief
Biotropika: Journal of Tropical Biology
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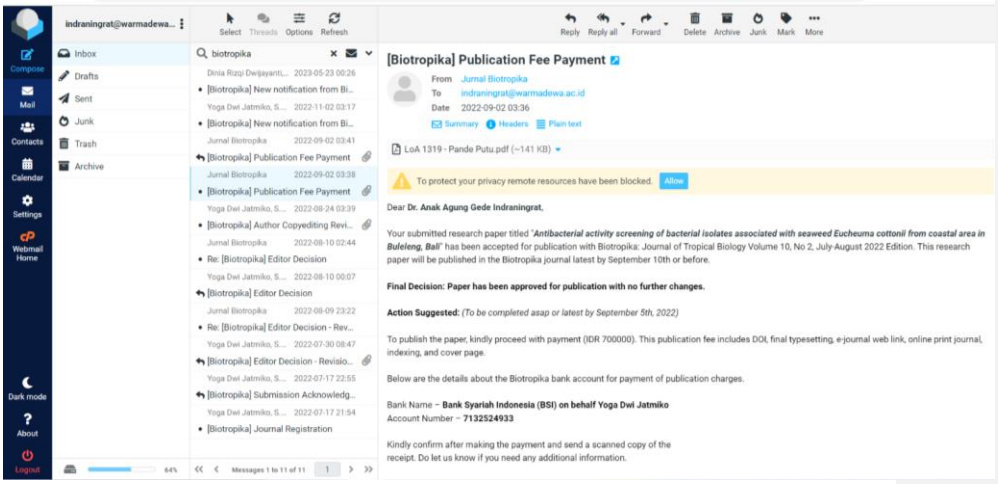
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Dear Dr. Anak Agung Gede Indraningrat,
Your submitted research paper titled "Antibacterial activity screening of bacterial isolates associated with seaweed Eucheuma cottonii from coastal area in Buleleng, Bali" has been accepted for publication with Biotropika: Journal of Tropical Biology Volume 10, No 2, July-August 2022 Edition. This research paper will be published in the Biotropika journal latest by September 10th or before.
Final Decision: Paper has been approved for publication with no further changes.
Action Suggested: (To be completed asap or latest by September 5th, 2022)
To publish the paper, kindly proceed with payment (IDR 700000). This publication fee includes DOI, final typesetting, e-journal web link, online print journal, indexing, and cover page.
Below are the details about the Biotropika bank account for payment of publication charges.
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