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ANTAGONISTIC ACTIVITY OF MARINE BACTERIA FROM KARIMUN ISLAND, INDONESIA

[*Aktivitas Antagonistik Bakteri Laut dari Pulau Karimun, Indonesia*]

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ABSTRACT

Antimicrobial resistance is becoming a major global crisis in the public healthcare system. Discovering new antimicrobial compounds from various sources and places is an alternative way to resolve this problem. Marine bacteria were known as a new promising source of bioactive compounds. Therefore, in this study, we aim to isolate the marine bacteria from sediment and seawater and test their antagonistic activity against pathogenic bacteria using the disk diffusion method. The active isolates were then identified based on the 16S rDNA sequence. A total of 65 bacterial isolates have been successfully isolated from the seawater and marine sediment from Karimun Island and, 12 isolates showed activity against tested pathogenic bacteria. Seven, two and one isolates againsted *B. subtilis, S. aureus, and E. coli, respectively.* The 16S rDNA sequences showed that the selected isolates belong to *Cellulosimicrobium funkei, Gordonia* sp., *Kocuria salina, Micrococcus aloeverae, Micromonospora aurantiaca, Mumia* sp., *Nocardioides* sp and *Pseudoalteromonas shioyasakiensis*. Most of the isolates with antibacterial activity were identified as Actinobacteria and one isolate from the Gammaproteobacteria. Isolate KRSd2 (2) shared 97,79% identity with *Gordonia bronchialis*. Further, a taxonomical study of the isolate compared with known species and chemical analysis of bioactive compounds are needed.

Keywords: Marine bacteria, antimicrobial, sediment, seawater

ABSTRAK

Resistensi antimikroba menjadi krisis global utama pada sistem kesehatan masyarakat. Salah satu cara untuk menyelesaikan masalah tersebut adalah dengan menemukan senyawa-senyawa antimikroba baru dari berbagai sumber dan lokasi. Bakteri laut dikenal sebagai sumber baru senyawa-senyawa bioaktif yang prospektif. Oleh karena itu, pada penelitian ini kami bertujuan untuk mengisolasi bakteri dari sedimen dan air laut dan menguji aktivitas antagonistiknya terhadap bakteri pathogen menggunakan metode difusi cakram. Isolat yang aktif selanjutnya diidentifikasi berdasarkan urutan gen 16S rRNA. Sebanyak 65 isolat bakteri telah berhasil diisolasi dari air dan sedimen laut dari Pulau Karimun, dan 12 isolat menunjukkan aktivitas terhadap bakteri uji.Tujuh, dua dan satu isolate aktif terhadap *B. subtilis, S. aureus,* dan *E. coli*, berturut-turut. Hasil sekuen 16S rDNA menunjukkan isolat terpilih adalah *Cellulosimicrobium funkei, Gordonia* sp., *Kocuria salina, Micrococcus aloeverae, Micromonospora aurantiaca, Mumia* sp., *Nocardioides* sp dan *Pseudoalteromonas shioyasakiensis*. Isolat yang memiliki aktivitas antibakteri diidentiifikasi termasuk kedalam kelompok Aktinobakteria dan satu isolat dari Gammaproteobacteria. Isolat KRSd2(2) memiliki kemiripan sebanyak 97.79% dengan *Gordonia bronchialis*. Maka dari itu, diperlukan studi taksonomi lebih lanjut dari isolat tersebut dibandingkan dengan spesies yang telah diketahui dan analisis kimia senyawa bioaktifnya.

Kata kunci: Bakteri laut, antimikroba, sedimen, air laut

INTRODUCTION

Antimicrobial resistance (AMR) is a serious threat to the world's public health system (Ferri *et al.,* 2017; Saputra *et al.,* 2017). During the COVID-19 pandemic, antibiotic prescription to treat the patient has changed. The use of broad-spectrum antibiotics, self-prescribed antibiotics, and misinformation lead to the abuse of antibiotics (Founou *et al.,* 2021; Toro-Alzate *et al.,* 2021). The antibiotic misuse in humans and animals was responsible for accelerating the number of AMR strains. Infection with AMR pathogens will increase the risk of illness and prolonged medication and burden healthcare costs (Saputra *et al.,* 2017; Yassin *et al.,* 2017; Huy *et al.,* 2021).

In 2017, World Health Organization (WHO) released the list of pathogens, including *Acinetobacter baumanii, Enterobacter* species, *Enterococcus faecium, Klebsiella pneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa* as pathogen strains with high priority for new antimicrobial development (Mulani *et al.,* 2019). The development and commercialization of novel antibiotics have lagged since the 1990s. A total of 11 new antimicrobial compounds were approved by the United States Food and Drug Administration between 2017 and 2019 (De Oliveira *et al.,* 2020). The development of new antimicrobial has been conducted from various resources, including plants, insects, reptiles, mammals, fungi, and bacteria from aquatic and terrestrial ecosystems (Ali *et al.,* 2018).

The marine environment consists of specific habitat affected by a wide range of temperature, salinity, and hydrostatic pressure and a source for a diverse plethora of life with unique structural, functional and metabolic properties and an emerging source for natural products. Marine microorganisms could survive in all oceanic stratification. However, the culturing technique and poorly defined taxonomy became a significant challenge in developing marine biodiscovery (Joint *et al.,* 2010; Ameen *et al.,* 2021). From 2017 to 2018, the total number of natural products isolated from marine bacteria, microalgae, fungi, plants, and

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animals as promising compounds with pharmaceutical activities reached about 4,534 compounds (Carroll *et al.,* 2020). Between 2010 and 2015, 52 active compounds from marine bacteria were reported, and 80% of the active compounds were recognized as new compounds. About 22 new active compounds showed antibacterial activity, and most of them were isolated from Actinobacteria and Bacilli (Schinke *et al.,* 2017). Antagonistic activity of the marine bacteria is the screening method to assess the reduction of pathogenic bacteria through releasing antibacterial compounds. Indonesia is the largest archipelagic country with more than 17,000 islands. This geographical characteristic was resulting Indonesia as a mega-biodiversity of marine organisms. The highly diverse marine organism will produce various metabolites with unique chemical properties (Abranches, 2020).

Considering that, this study will investigate marine bacteria's antagonistic activity isolated from Karimun Island, Indonesia, against pathogenic bacteria using the disk diffusion method. In addition, the potential isolate would provide a new source of antibiotics against AMR bacteria.

MATERIALS AND METHODS

Collection of samples and Isolation of marine bacteria

Four sediments (KRSd) and two seawater (KRW) samples were collected from 6 sites nearshore on Karimun island. Sediment and water samples were collected into a 50 mL sterile falcon bottle. For the sediment, the samples were taken about 5 cm below the surface using a sterile shovel. Marine bacteria were isolated using the serial dilution method. About 1 g sediment or 1 mL seawater samples were diluted to 10^{-1}, 10^{-2} and 10^{-3}. A volume of 200 µL of the sample was transferred to 1/5 NBRC 802 added with 2.5% NaCl, 0.005% *cycloheximide,* and 0.002% *nalidixic acid* (Hamada *et al.,* 2013) and seawater agar supplemented with 10mM (Joint *et al.,* 2010). The inoculated medium was incubated at 30°C for two weeks until marine bacteria colonies grew on the plates. The isolates were then transferred into a new NBRC 802 medium to obtain pure cultures. Cultures were stored as glycerol stocks at -80°C for further experiment.

Marine bacteria cultivation and test isolates preparation

Marine bacteria were cultivated in Peptone yeast extract (PYE) agar with 2% NaCl (Peptone 10g/L, yeast extract 2g/L, $MgSO_4.7H_2O$ 1g/L, NaCl 20g/L, agar 20 g/L) at 30°C. The plates were incubated at 30°C for four days. Three test isolates were selected to represent gram-negative and gram-positive bacteria, including *Bacillus subtilis* InaCC B1, *Escherichia coli* InaCC B5, and *Staphylococcus aureus* InaCC B3. These test isolates were maintained in Nutrient Agar (NA) at 37°C.

Antagonistic activity of marine bacteria

For antagonistic activity assays, the marine bacteria were inoculated into peptone yeast extract broth with 2% NaCl and incubated at 30°C, 140 rpm for four days. Next, the test isolates were inoculated into Nutrient Broth (NB) and incubated at 30°C with agitation at 140 rpm for 18h. Finally, an antagonistic activity assay was carried out using the disk diffusion method. About 1% of the test isolate suspension was mixed with 50 mL semi-solid NA (approximately 10^6 CFU/ml), and 4 ml of the mixture was poured onto PYE agar. After the agar solidified, a 7 mm filter paper disk was placed onto the agar, and 10 µl of marine bacteria suspension was dropped onto the filter paper disk. The agar plates were incubated at 30 °C for 24h.

The antagonistic activity was observed by the formation of a clear zone around the filter paper disk and calculated as Clear Zone Index (CZI) according to equation (1) (Ratnakomala *et al.,* 2016).

$$CZI = \frac{clear\ zone\ diameter - filter\ paper\ diameter}{filter\ paper\ diameter} \quad (1)$$

Identification of marine bacteria

The selected marine bacteria that showed antagonistic activity were identified by comparing the 16S rRNA gene with the 16S rRNA gene in the EzBiocloud database. The genomic DNA from the marine bacteria was prepared using the boiling method (Bansal *et al.,* 1996). Then, the 16S rDNA was amplified by PCR using primer 27F and 1492R (Weisburg *et al.,* 1991) with the following conditions: initial denaturation at 95 °C for 5 min, 35 cycles of amplification (20 s at 95 °C, 45 s at 50 °C, 1.5 min at 72 °C), and a final extension at 72 °C for 10 min. Sequencing of the PCR products was performed using an ABI 3730xl DNA analyzer by Macrogen Inc (Seoul, South Korea).

The obtained sequences were trimmed, assembled, and compared with the 16S rDNA of bacterial type strain database using the EzBioCloud server (Yoon *et al.,* 2017). The DNA sequences with high similarity and neighbor species were picked for phylogenetic analysis. The phylogenetic tree reconstruction was carried out according to (Setiawan and Larasati, 2019).

RESULTS

Isolation of marine bacteria

A total of 65 isolates of marine bacteria were successfully collected from sediment and seawater on Karimun Island (Table 1). Forty-six were isolated from sediment samples and nineteen from seawater samples.

Table 1. A number of marine bacteria were isolated from sediment samples of Karimun Island. (*Sejumlah bakteri laut diisolasi dari sampel sedimen Pulau Karimunjawa*).

No	Sample code	Location		Source	Temperature (°C)	pH	Number of isolates
		Lat	Long				
1	KRSd1	1.13669	103.39125	Sediment	31	7	12
2	KRSd2	1.13669	103.39125	Sediment	31	7	19
3	KRSd3	1.13736	103.39069	Sediment	33	7	10
4	KRSd4	1.13755	103.39061	Sediment	28	5	5
5	KRW1	1.09091	103.38108	Seawater	32	7	8
6	KRW2	1.12750	103.39369	Seawater	31	7	11
		Total					65

Antagonistic activity of marine bacteria

A total of 65 marine bacteria isolates collected from Karimun Island, Kepulauan Riau, Indonesia, were screened for antagonistic activity against *B. subtilis*, *E. coli* and *S. aureus* using the disk diffusion method. From the screening results, it was revealed that 12 isolates showed an inhibition effect against test bacteria. Out of 12 isolates with inhibition effects, seven isolates inhibit *B. subtilis,* one isolate inhibits *E. coli*, two isolates inhibit *S. aureus*, one isolate inhibits *B. subtilis* and *S. aureus*, and one isolate inhibits *E. coli* and *S. aureus*. Of all the isolates, no isolates can inhibit all test bacteria.

Antagonistic activities of marine bacteria against test isolates. (Aktivitas antagonis bakteri laut terhadap isolat uji).

No	Isolate codes	Clear zone index		
		B. subtilis	*E. coli*	*S. aureus*
1	KRW1(2.6)	0.21	-	-
2	KRW2(2.5)	0.79	-	-
3	KRW2(4.3)	-	-	0.14
4	KRSd1(2.6)	-	-	0.14
5	KRSd2(3.4)	0.21	-	-
6	KRSd2(1.4)	0.21	-	-
7	KRSd2(1)	0.21	-	-
8	KRSd2(2)	0.21	-	0.28
9	KRSd2(7)	-	0.43	0.28
10	KRSd2(9)	-	0.29	-
11	KRSd2(10)	0.42	-	-
12	KRSd2(3)	0.18	-	-

Isolates KRW2(2.5) have the highest antagonistic activity against *B. subtilis* (Figure 1. A), followed by KRSd2(10) with the CZI values 0.79 and 0.42, respectively. The growth of *E. coli* was inhibited by isolates KRSd2(7) with a CZI value of 0.43 (Figure 1. B). Isolates KRSd2(2) inhibited the growth of *S. aureus* with CZI values of 0.28 (Figure 1. C).

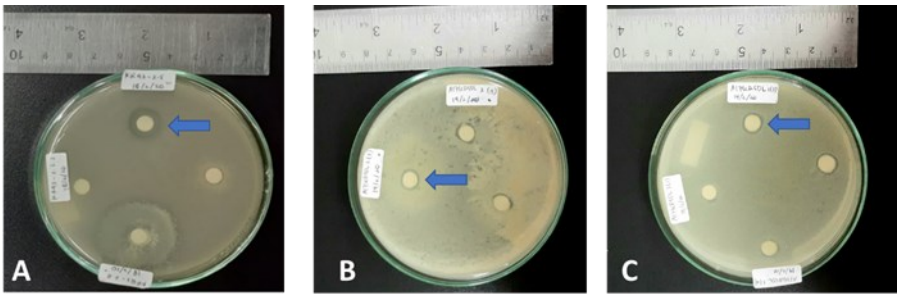

Figure 1. Clear zones produced by the antagonistic activity (in arrow) of isolate KRW2(2.5) against *B. subtilis* (A), isolate KRSd2(7) against *E. coli* (B), isolate KRSd2(2) against *S. aureus* (C). (*Zona bening yang dihasilkan oleh aktivitas antagonis (panah) isolat KRW2(2.5) terhadap B. subtilis (A), isolat KRSd2(7) terhadap E. coli (B), isolat KRSd2(2) terhadap S. aureus (C)*).

Identification of marine bacteria with antagonistic activities

Marine bacteria were identified using the 16S rRNA gene and compared with the strain type from the EzBioCloud database. A total of twelve isolates were sequenced, but only ten isolates give a good sequence quality and the phylogenetic tree from those isolates was reconstructed. Most isolates were identified as Actinobacteria and one isolate from the Gammaproteobacteria. About eight genera were obtained based on a similarity search, including *Cellulosimicrobium, Gordonia, Kocuria, Micrococcus, Micromonospora, Mumia, Nocardioides* and *Pseudoalteromonas*. In addition, three isolates belonged to the species *Micrococcus luteus* supported by a high similarity value, including KRW2(4.3), KRSd1(2.6), and KRSd2 (3.4).

Identification of marine bacteria based on 16S rRNA gene similarity. (*Identifikasi bakteri laut berdasarkan kemiripan gen 16S rRNA*).

No	Isolate codes	Length of obtained 16S rRNA gene (bp)	Hit taxon	Accession	Similarity (%)	Class
1	KRSd1(4)	1362	*Cellulosimicrobium funkei*	AY501364	99.34	Actinobacteria
2	KRSd2(2)	1362	*Gordonia bronchialis*	X53201	97.79	Actinobacteria
3	KRW1(2.6)	1323	*Kocuria salina*	LT674162	99.62	Actinobacteria
4	KRW2(4.3)	1312	*Micrococcus luteus*	AF542073	99.39	Actinobacteria
5	KRSd1(2.6)	1337	*Micrococcus luteus*	AF542073	99.55	Actinobacteria
6	KRSd2(3.4)	1339	*Micrococcus luteus*	AF542073	99.55	Actinobacteria
7	KRSd2(3)	1359	*Micromonospora aurantiaca*	X92604	100.00	Actinobacteria
8	KRSd2(10)	1371	*Mumia xiangluensis*	KT220418	98.24	Actinobacteria
9	KRSd2(1)	1349	*Nocardioides cavernae*	KX815990	98.81	Actinobacteria
10	KRW2(2.5)	1361	*Pseudoalteromonas shioyasakiensis*	AB720724	99.19	γ-Proteobacteria

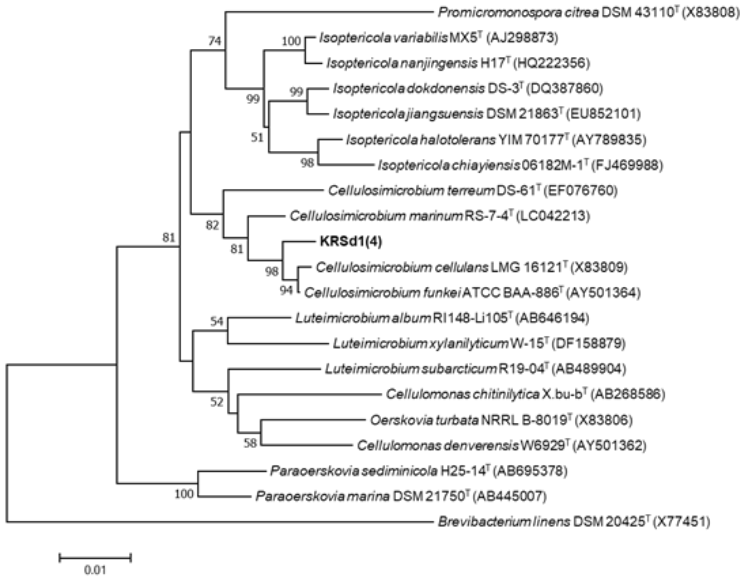

Figure 2. Neighbor-joining tree constructed from the 16S rRNA gene from isolate KRSd1(4) (shown in bold) and the related sequence with accession number shown in parentheses. Only bootstrap value > 50% (n=1000) were shown in node. (*Neighbor-joining tree yang dibangun dari gen 16S rRNA dari isolat KRSd1(4) (dicetak tebal) dan sekuens terkait dengan nomor aksesi ditampilkan dalam tanda kurung. Hanya nilai bootstrap > 50% (n=1000) yang ditampilkan di node*).

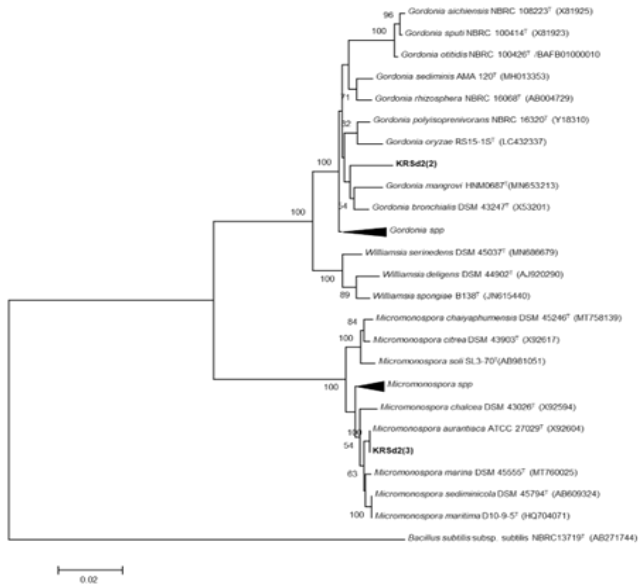

Figure 3. Neighbor-joining tree constructed from the 16S rRNA gene from isolates KRSd2(2) and KRSd2(3) (shown in bold) and the related sequence with accession number is shown in parentheses. Only bootstrap value > 50% (n=1000) were shown in node. (*Neighbour-joining tree yang dibangun dari gen 16S rRNA dari isolat KRSd2(2) dan KRSd2(3) (dicetak tebal) dan sekuens terkait dengan nomor aksesi ditampilkan dalam tanda kurung. Hanya nilai bootstrap > 50% (n=1000) yang ditampilkan di node*).

The isolate KRSd1(4) was nested with *Cellulosimocrobium marinum* supported with a high bootstrap value (Figure 2). The phylogram showed that the isolate KRSd1(4) belongs to *C. funkei* based on the sum of branch length of each strain in the clade. The phylogram of isolates KRSd2(2) and KRSd2(3) revealed that the position of the isolate was nested in the *Gordonia* clade and the *Micromonosprora aurantiaca*, respectively (Figure 3). The bootstrap value of the KRSDL2(2) clade has a < 50% support value, making this clade not reliable for identifying the isolate up to species level. The similarity search showed that the similarity value with *Gordonia bronchialis* was about 97%. Therefore, the isolate KRSd2(2), identified as *Gordonia* sp. Meanwhile, the isolate KRSd2(3) was identified as *M. aurantiaca* based on the phylogram and the similarity value.

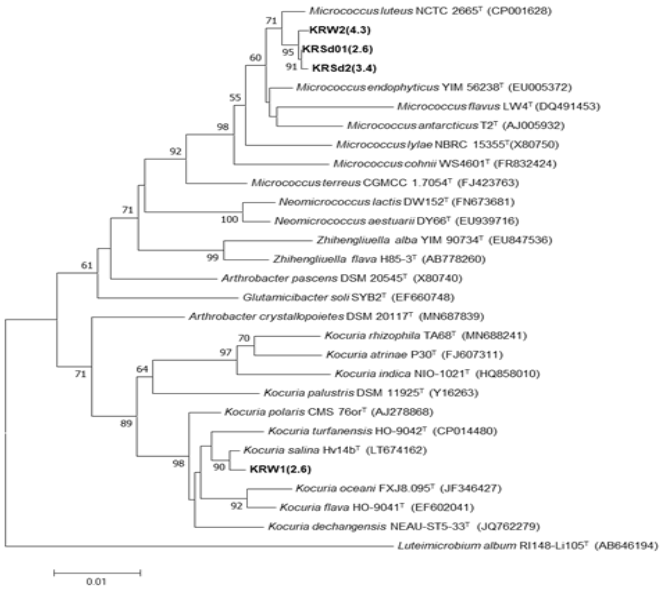

Figure 4. Neighbor-joining tree constructed from the 16S rRNA gene from isolates KRW1(2.6), KRW2(4.3), KRSd1(2.6), and KRSd2(3.4) (shown in bold), and the related sequence with accession number shown in parentheses. Only bootstrap value > 50% (n=1000) were shown in node. (*Neighbour-joining tree yang dibangun dari gen 16S rRNA dari isolat KRW1(2.6), KRW2(4.3), KRSd1(2.6), dan KRSd2(3.4) (dicetak tebal), dan sekuens terkait dengan nomor aksesi ditunjukkan pada tanda kurung. Hanya nilai bootstrap > 50% (n=1000) yang ditampilkan di node*).

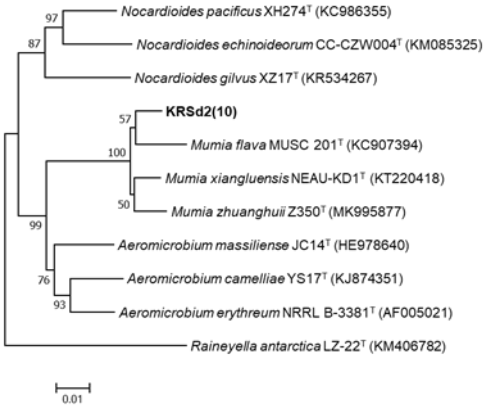

Figure 5. Neighbor-joining tree constructed from the 16S rRNA gene from isolate KRSd2(10) (shown in bold) and the related sequence with accession number shown in parentheses. Only bootstrap value > 50% (n=1000) were shown in node. (*Neighbor-joining tree yang dibangun dari gen 16S rRNA dari isolat KRSd2(10) (dicetak tebal) dan sekuens terkait dengan nomor aksesi ditampilkan dalam tanda kurung. Hanya nilai bootstrap > 50% (n=1000) yang ditampilkan di node*).

The phylogenetic reconstruction revealed that the isolates KRW2(4.3), KRSd1(2.6), and KRSd2 (3.4) were nested in the same clade with *Micrococcus luteus* and supported by a high (> 70%) bootstrap value (Figure 4). The isolate KRW1(2.6) was nested in the same clade with *Kocuria turfanensis* and *K. salina* (Figure 4). That isolate shared the most common ancestor with *K. salina* supported with a 90% bootstrap value (Figure 4). The reconstruction of the phylogenetic tree of the isolate KRSd2(10) (Figure 5) showed that the isolate was nested in the same clade with all Mumia-type strains and designated as *Mumia* sp.

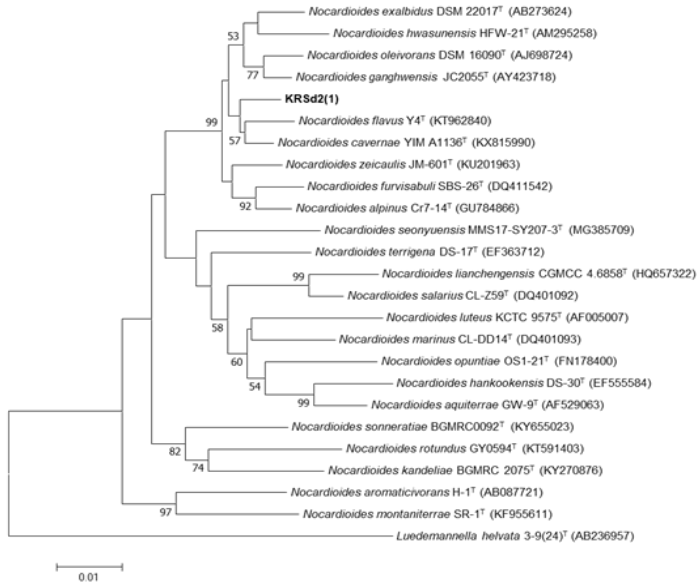

Figure 6. Neighbor-joining tree constructed from the 16S rRNA gene from isolate KRSd2(1) (shown in bold) and the related sequence with accession number shown in parentheses. Only bootstrap value > 50% (n=1000) were shown in node. (*Neighbour-joining tree yang dibangun dari gen 16S rRNA dari isolat KRSd2(1) (dicetak tebal) dan sekuens terkait dengan nomor aksesi ditampilkan dalam tanda kurung. Hanya nilai bootstrap > 50% (n=1000) yang ditampilkan di node*).

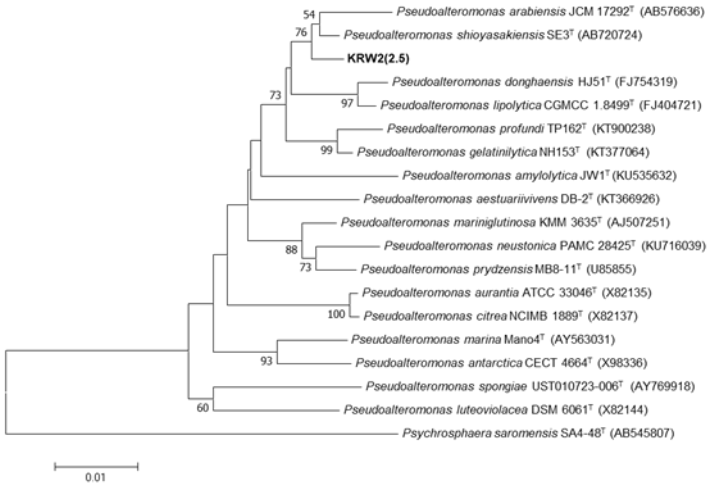

Figure 7. Neighbor-joining tree constructed from the 16S rRNA gene from isolate KRW2(2.5) (shown in bold) and the related sequence with accession number shown in parentheses. Only bootstrap value > 50% (n=1000) were shown in node. (*Neighbor-joining tree yang dibangun dari gen 16S rRNA dari isolat KRW2(2.5) (dicetak tebal) dan sekuens terkait dengan nomor aksesi ditampilkan dalam tanda kurung. Hanya nilai bootstrap > 50% (n=1000) yang ditampilkan di node*).

The phylogram of isolate KRSd2(1) showed the isolate was nested in the same clade with *Nocardioides flavus* and *N. cavernae*. However, the clade was not supported with a reliable bootstrap value (< 70%) (Figure 6). Therefore, the isolate KRSd2(1) belonged to *Nocardioides* sp. The reconstruction of the phylogenetic tree of the isolate KRW2(2.5) shows the isolate nested in the clade of *Pseudoalteromonas*. Analysis of the phylogram showed that isolate KRW2(2.5) belongs to *Pseudoalteromonas shioyasakiensis* due to the shortest distance of the isolate with the type species in the clade and supported with > 70% bootstrap value (Figure 7). The phylogenetic reconstruction showed some of the isolates separate from the clade of the nearest strain with a long branch length, including isolates KRSd1(4), KRSd2(1) and KRSd2(2). Further, a taxonomical study of these isolates compared with known species is needed.

DISCUSSION

Marine ecosystems reserve copious bioactive activities from marine species. The marine biosphere consists of diverse groups of microorganisms with unique metabolic, functional and structural properties (Schinke *et al.,* 2017; Carroll *et al.,* 2020). Unique chemical ecologists in the marine ecosystem might influence the evolution of secondary produces by marine microorganisms. As the predominant species, marine bacteria show a capability to produce natural products with various biological activities, including antimicrobial compounds.

This study revealed the diversity of culturable marine bacteria from marine sediment and seawater of Karimun island and itsantibacterial activity. A total of 65 isolates have been isolated from marine sediments and seawater. The media used for the isolation were designated to give the salinity as seawater. That condition will enhance the growth of marine bacteria, in particular Actinobacteria and Proteobacteria (Joint *et al.,* 2010; Hamada *et al.,* 2013). Marine bacteria can be found in all types of niches in marine ecosystems, such as seawater, sediment, and hydrothermal vent, and are also associated with marine organisms, including sponges, coral, and vertebrates (Jensen *et al.,* 2005; Joint *et al.,* 2010; Andrianasolo *et al.,* 2012; Freel *et al.,* 2012; Bech *et al.,* 2020). The sediment used as a source in this study is the nearshore sediments. Studies using nearshore sediment have revealed that many strains found in these samples are closely related or identical to strains previously observed from land (You *et al.,* 2005; Prieto-Davó *et al.,* 2008).

A total of 12 isolates showed antagonistic activity against test bacteria, and ten isolates have been identified, with 9 isolates identified as Actinobacteria and one isolate identified as Proteobacteria. Actinobacteria is a well-known producer of natural products. Population sizes of Actinobacteria, in particular, actinomycetes in ocean sediment, have been shown to vary with physicochemical parameters including temperature, pH, pressure, total organic carbon, and salinity, the preferred levels of these parameters varying with location (Ghanem *et al.,* 2000). Marine actinomycetes were the major source of marine natural products. About 70% of antibacterial compounds reported during 2010–2015 were produced by Actinobacteria (Schinke *et al.,* 2017).

The members of Actinomycetales, particularly isolated from the marine ecosystem, contain an incredible diversity in phenotypic/genomic characteristics and utility in various fields. For instance, within the medical field it has been utilized as a source of secondary metabolites that function as antibiotics, antifungals, anthelmintics, and antitumor agents (Jagannathan *et al.,* 2021). Therefore, as more marine actinomycetes are discovered and investigated, particularly rare isolates that are understudied, it is expected that more natural products' potential for antibiotics and other medical treatments will be identified.

Several previous studies have reported that some marine bacteria isolated from marine sediment such as *Verrucosispora*, *Micromonospora harpali, Streptomyces*, *Saccharomonaspora, Actinomadura, Glycomyces, Nocardia, Variovorax* have antimicrobial activities against different kinds of microorganisms (Schinke *et al.,* 2017; Qi *et al.,* 2020; Chen *et al.,* 2021). In addition, several marine actinobacteria produce structurally unique secondary metabolites. For example, *Streptomyces* sp. isolated from marine sediment produces heronamycin A and moderates antimicrobial activity against two different strains *B. subtilis* (Raju *et al.,* 2012). Novel antimicrobial compounds from a *Streptomyces* sp. isolated from marine sediment, including three benzopyrone derivatives, 7-methylcoumarin, and two flavonoids, rhamnazin and cirsimaritin (El-Gendy *et al.,* 2008).

Isolate KRW2(2.5) was given the highest antagonistic activity against *B. subtilis*, followed by isolate KRSd2(10). Those isolates were identified as *P. shioyasakiensis* and *Mumia* sp., respectively. Marine γ-Proteobacteria members of the *Pseudoalteromonas* are also found associated with marine invertebrates and seaweed (Bowman, 2007; Offret *et al.,* 2016). The genus of marine *Pseudoalteromonas* is a natural product reserve with various activities against Gram-positive and Gram-negative bacteria, including antibacterial peptides and predator-prey interaction (Desriac *et al.,* 2013; Bibi *et al.,* 2018; Tang *et al.,* 2020).

Murphy et al. (2014) reported the biosynthesis

of thiomarinol A derived from *Pseudoalteromonas* sp. that is effective against methicillin-resistant *Staphylococcus aureus*. Also, Whalen et al. (2015) reported that *P. piscicida* produced the known 3,4-dibromopyrrole-2,5-dione that inhibits the efflux pump of *Enterobacteriaceae* and *Pseudomonas aeruginosa*. In addition, many other compounds produced by macroalgae-associated bacteria of the genus *Pseudoalteromonas* have shown antimicrobial activity, including 2,4-dibromo-6-chlorophenol (Jiang *et al.,* 2000), korormicin (Goecke *et al.,* 2010), violacein (Matz *et al.,* 2008).

The 16S rRNA sequences show that the most identified bacterial isolates belonged to Actinobacteria and were compromised of seven different genera, including *Cellulosimicrobium*, *Gordonia, Kocuria, Micrococcus, Micromonospora, Mumia,* and *Nocardioides*. The actinomycetes are one of great diversity that produces secondary metabolites. Moreover, secondary metabolites produced by Actinobacteria are an abundant source of antibiotics. Therefore, marine actinobacteria is a well-known secondary metabolite producer (Schinke *et al.,* 2017). Although those isolates do not belong to the prolific genus of the Actinobacteria, *Streptomyces*, they hold the potential to produce natural products (Santos *et al.,* 2019; Shamikh *et al.,* 2020).

No study reported that *Cellulosimicrobium funkei* had antibacterial activity. However, Kim et al. (2020) reported that *C. funkei* HY-13 KCTC 11302BP was isolated from the intestine of the earthworm *Eisenia fetida*. This bacterium is one of the representative fibrolytic gut bacteria and possesses at least six endotype glycoside hydrolases showing peculiar biocatalytic activities toward cellulosic and hemicellulosic polysaccharides.

CONCLUSION

The present study investigates the antagonistic activity of marine bacteria isolated from Karimun Island, Indonesia. About 18% of the marine bacteria isolates showed antagonistic activity against at least one test bacteria using the disk diffusion method. The antagonistic bacteria were identified using the 16S rRNA gene. Phylogenetic reconstruction of the antagonistic bacteria revealed the marine bacteria as *Micrococcus luteus, Kocuria salina, Cellulosimicrobium funkei, Nocardioides* sp., *Mumia* sp., *Gordonia* sp., *Micromonospora aurantiaca* and *Pseudolateromonas shioyasakiensis*. The isolate KRW2(2.5) has the highest antagonistic activity against *B. subtilis* and was identified as *P. shioyasakiensis*. The phylogenetic reconstruction showed that some of the isolates that have antagonistic activity separate from the clade of the nearest strain with a long branch length. Further, a taxonomical study of the isolates compared with known species is needed. Also, the antagonistic activities of isolated marine bacteria show weak activities against test bacteria. Moreover, there are no isolates that could inhibit all test bacteria.

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