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# Potential of marine sponge *Jaspis* sp.-associated bacteria as an antimicrobial producer in Enggano Island

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**ABSTRACT** Sponges, a group of marine multicellular animals with a porous body structure, show potential for the production of bioactive compounds. Sponge-associated bacteria are an alternative antimicrobial producer due to their high content of bioactive compounds. This study aimed to identify the highest-potential antimicrobial-producing bacteria isolate associated with *Jaspis* sp. sponges from Enggano island. The isolated bacteria were screened for antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida albicans* using cultures, supernatants, pellets, and crude extracts. The study also conducted genetic identification to determine the identity of the isolate with the greatest potency and its closest relationship using the 16S rRNA gene. The antimicrobial activity was determined by monitoring and measuring the diameter of the formed clear zones. The results of the observations of morphological characteristics revealed nine isolates from *Jaspis* sp. that each consisting of 6 JABS isolates and 3 JABB isolates. Based on isolates that had antimicrobial activity, JABS6 isolates had the best antimicrobial activity, with the diameter of inhibition zones of 24.7, 8.2, 4.6, and 33.7 mm for *E. coli*, *P. aeruginosa*, *S. aureus* and *C. albicans*, respectively. The genome sequencing of the JABS6 isolate confirmed that it was identical to *Bacillus thuringiensis* strain USS-CAP-1. The study concludes that this finding shows promise for the further development of future antimicrobial agents.

**KEYWORDS** antimicrobial; symbiont bacteria; Enggano island; *Jaspis* sp.; screening.

## 1. Introduction

A marine ecosystem has caught the scientists' attention due to its high natural resources and relationships among all organisms. Blockley et al. (2017) suggested that about 89% of marine ecosystems belong to the invertebrate group with a total of 174,600 species identified. Invertebrates are a group of organisms capable of symbiosis with other organisms, one of which is microbes. Invertebrates with a symbiosis with microbes are able to produce bioactive compounds, so the prospects are promising because of the increased resistance of pathogenic microbes to antibiotics, such as bacteria and fungi. Therefore, looking for new alternative drugs with more potential is necessary. Marine microorganisms are well known for their potential to produce active compounds, especially bacteria. In addition, marine bacterial growth is generally supported by the symbiont formation pattern with the benthic marine organisms, such as sponges, due to the lack of nutrients in the ocean environment (Abubakar et al. 2012).

Sponges, one of the phylum Porifera animals, are one of the oldest Metazoa and are considered important in the evolutionary process. They are also the most abundant source of producing bioactive compounds, so they are of pharmaceutical relevance (Conkling et al. 2019). Their body consists of many pores, and there are aquiferous canals mainly for the entry of seawater. Sponges also lack organs, muscles, and a nervous system (Riesgo et al. 2014). Instead, they feed through the filtration of organic matter and organism debris in the ostium. Because of their ability as an efficient filter feeder, any symbiont can swimmingly colonize sponges. Moreover, sponges also provide shelter for microorganisms, such as unicellular algae, cyanobacteria, heterotrophic bacteria, facultative anaerobic bacteria, and archae bacteria, considered microbial fermenters. Correspondingly, the symbiotic relationships between sponges and all microbial symbionts deliver support and protection to both, as well as provide necessary nutritional requirements (Taylor et al. 2007). This is also supported by Hentschel et al. (2002) that those microbial symbionts play a role significantly as bioactive compounds producers in the form of the secondary metabolites con-

tributing to the pharmaceutical value products-antibiotics, anticancer, antiviral, and antimicrobial, for instance (Murniasih 2003).

Many prior studies exploring the antimicrobial activity from sponges-associated bacteria have been reported, such as Abubakar et al. (2012), who successfully found 32 (45.71%) and 20 (29.41%) isolates from mesohil and surface of the sponges, respectively. That discovery showed that those obtained bacterial isolates were able to inhibit some pathogenic microbial growth, namely *Pseudomonas aerugenosa*, *Staphylococcus aureus*, *Vibrio harveyii*, *Escherichia coli*, *Candida albicans*, and *C. tropicalis*. Based on the partial identification, most of the isolates were similar to *Bacillus*. Moreover, ten isolates discovered from the Ternate Island actively produced the secondary metabolites and strongly inhibited the growth of *Klebsiella pneumonia*, *S. aureus*, *P. aeruginosa*, *B. subtilis*, *Salmonella thypi*, and *E. coli* (Trianto et al. 2019). Likewise, *Haliclona (Reniera)* sp. which provided bacterial isolate strain PSP. 39-04 from Panjang Island, Jepara also showed antimicrobial (Asagabaldan et al. 2017). This isolate was closely related to Chromohalobacter salixigens strain DMS3043 and strongly resisted *P. aeruginosa*, *S. aureus*, and *Acinotebacter baumannii*.

Enggano is one of the outermost islands located in Bengkulu province and directly encounters the Hindia Ocean. It has a considerable ecosystem that provides vast organic matter for living organisms (Ratnakomala et al. 2016). Based on the study by Senoaji (2003), Enggano has an encouraging potential to be developed as a tourism and natural resource, including *Jaspis* sp. sponges. Nevertheless, exploring potential natural resources in the Enggano environment has never been studied optimally. For this reason, this research expected to obtain *Jaspis* sp. sponges containing the antimicrobial compound-producing bacteria symbionts.

## 2. Materials and Methods

### 2.1. Samples collection and bacteria isolation

The sponges belonging to *Jaspis* sp. were collected from Enggano island in April 2018. Administratively, Enggano is a subdistrict of North Bengkulu Regency with an approximate length and width area of 22 and 9.9 miles, respectively. The *Jaspis* sp. sponges were collected from Banjar Sari (05°17.404'S 102°09.866'E) and Bak Blau beach in Meok (05°19.141'S 102o13.470'E) village (Figure 1) using purposive sampling method at about 2 m depth. The isolation, purification, and morphological identification were performed in the Laboratory of Microbiology and Biotechnology, University of Bengkulu. 1 g of fresh sponges was cut, sterilized by seawater, and crushed. The sample was then serially diluted from $10^{-1}$ to $10^{-3}$ using sterile distilled water. Following that, the diluted sample was inoculated to SWC agar media using the spread plate method and incubated at 37 °C for 48 h. The well-proliferated isolates were separated based on the morphological features (shape, color, edge, elevation, and surface) and purified.

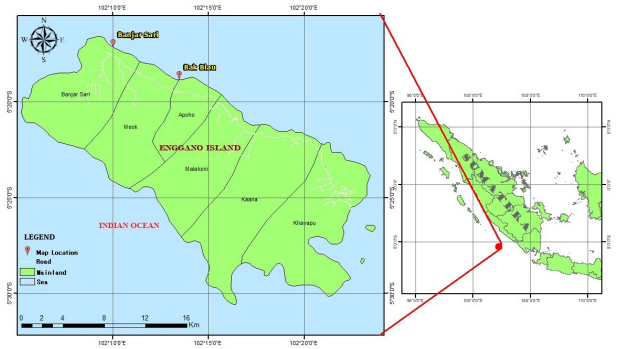
### 2.2. Antimicrobial-producing isolate screening against the pathogenic microbes

The microbe isolates having antimicrobial activities were determined by their phenotype. Different microbial phenotypes under antibiotic exposure or pathogenic microbes would then determine the level of susceptibility, resistance, tolerance, and persistence (Brauner et al. 2016). Firstly, this stage used bacterial cultures to be monitored in which a colony of each bacterial strain was spotted on the surface of pathogenic microbes cultures-containing Tryptic Soy Agar (TSA) media. In addition, supernatants, as well as pellets, the isolates to be used for the antimicrobial activity test were cultured on SWC liquid media (starter) for 24 h. 1.5 mL of the suspension was centrifuged at 10000 rpm for 5 min. The pellets and supernatant from the centrifugation were separated into different tubes. The pellet was dissolved in ±150 µL of the supernatant to reach 10 times dilution from the initial volume of the starter. Then, 20 µL of culture supernatant and pellet were dropped onto sterile paper discs on SWC agar medium containing the target bacteria. The plates were incubated for 24 h at ±27 °C. A positive result is indicated by the presence of a clear zone around the paper disc (Wahyudi et al. 2018). In order to gain active compounds from the crude extract of active cells, the extraction method (Müller et al. 2004) was followed. A total of 25 mL (1%) bacterial suspension was used as a starter and inoculated into 500 mL of liquid SWC medium. The culture was incubated and shaken at 100 rpm for 72 h at ±27 °C. The bacterial culture was extracted by adding ethyl acetate solvent 1:1 (v/v). The solvent layer was separated and evaporated using a rotary evaporator at 42 °C to obtain a crude extract of antimicrobial compounds. The extracted compounds were then dissolved with dimethyl sulfoxide (DMSO) and tested with the Kirby-Bauer method. The antimicrobial activity was determined by monitoring and measuring the diameter of the formed clear zones. Inhibition zones were considered antibacterial or antifungal. Isolate with the largest clear zone was kept for further stages.

### 2.3. Bacterial strain identification

The isolate with the largest inhibition activity against the pathogens was subjected to 16S rRNA identification to determine its profile genetically and phylogenetic position. The genomic DNA of the bacterial strain was extracted (Sambrook and Russell 2001) using the bacterial isolation kit (Geneaid) following the manufacturer's protocols. The 63F (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1387R (5'-GGG CGG WTG GTA CAA GGC-3') primers (Marchesi et al. 1998) were utilized for the DNA amplification using polymerase chain reaction (PCR). It was initiated with initial denaturation (94 ºC, 4 min), denaturation (94 ºC, 45 s), annealing (55 ºC, 1 min), extension (72ºC, 1 min 10 s), and ended with post extension (72 ºC, 7 min), which was performed in 30 cycles. The PCR product was

(a)




(b)

**FIGURE 1** Sampling of sponges. (a) *Jaspis* sp.sponges collected from Banjar Sari and Bak Blau beach, Meok. Overall view of (b) Enggano and Sumatera island

analyzed by 1% agarose gel and visualized under UV light (Gel Document System Axygen). Afterward, the amplified DNA was sequenced and compared to the other bacteria registered in the National Center for Biotechnology Information (NCBI). Finishing these stages, the data were re-interpreted in MEGA 6.0, followed by constructing a phylogenetic tree using the Neighbor-Joining Tree method with 1000 × bootstrap value for determining its closest relatives.

## 3. Results and Discussion

### *3.1. Isolation of microbial symbionts*

In this study, two potential areas providing the habitat of the *Jaspis* sp. sponges were decided as sampling locations, namely Banjar Sari and Bak Blau. A total of 566 marine bacterial colonies were isolated from the *Jaspis* sp. found in both spots. For comparison, a number of 255 bacterial colonies were from Banjar Sari, while the other 311 were from Bak Blau. The name of the sample was based on the origin from which the sponge sample was obtained. JABS1 means *Jaspis* sp. Banjar Sari (sample serial number) were bacteria isolated from Banjar Sari Beach, and JABB1 means *Jaspis* sp. Bak Blau (sample serial number) were bacteria isolated from Bak Blau Beach. Based on the observation of their morphological characters, it certainly seemed that there were nine JABS isolates and three JABB isolates (Figure 2.). They were dominantly round and white.

### *3.2. Antimicrobial assay against pathogenic microbes*

Isolated bacteria were used for pathogenic bacteria and fungi resistance ability tests. It was judged by monitoring the formed clear zone around bacterial strains in the agar media. To initiate the first screening, the isolates that had been collected were spotted directly on the surface of pathogenic microbial cultures-containing agar media. Two of the nine isolates, namely JABS5 and JABS6, showed the inhibition activity as presented in Table 1 and Figure 3. JABS5 depicted inhibition of *S. aureus* growth with the diameter of clear zone of 6.7 mm, while JABS6 was detected against both *E. coli* and *P. aeruginosa* with the measured diameter of halo zone at consecutively 24.7 mm (strong) and 8.2 mm (moderate). Thus, isolates with inhibitory activity against pathogenic bacteria at the initial screening were further tested in the form of supernatants and pellets.

Supernatants and pellets collected from 24-hour culture were used for the active compound activity examination distributed within their cells. In the supernatant and pellet assays, both JABS5 and JABS6 isolates were retested using four pathogenic microorganisms, namely *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans*, in order to see the potential antimicrobial activity produced when using different assays. Antimicrobial activity of 2 selected isolates varied, in which JABS5 was able against Gram-positive and negative test strains (*S. aureus* and *P. aeruginosa*), while it inhibited neither *E. coli* nor *C. albicans*. Similar to JABS5, *S. aureus* growth was recorded to be resisted by pellets and supernatants of JABS6 isolate. Additionally, pellets extracted from JABS6 also played an inhibitory activity towards human pathogenic fungi, *C. albicans*, with 33.7 mm in diameter of clear zone (Figure 4), which was categorized as having a strong activity, interestingly. The strength or weakness of the inhibitory activity produced by bacterial cells is based on measuring the extent of the clear zone formed (Davis and Stout 1971). The diameter of the clear zone of 10-20 mm included activities with strong inhibition, the diameter of the clear zone of 5-

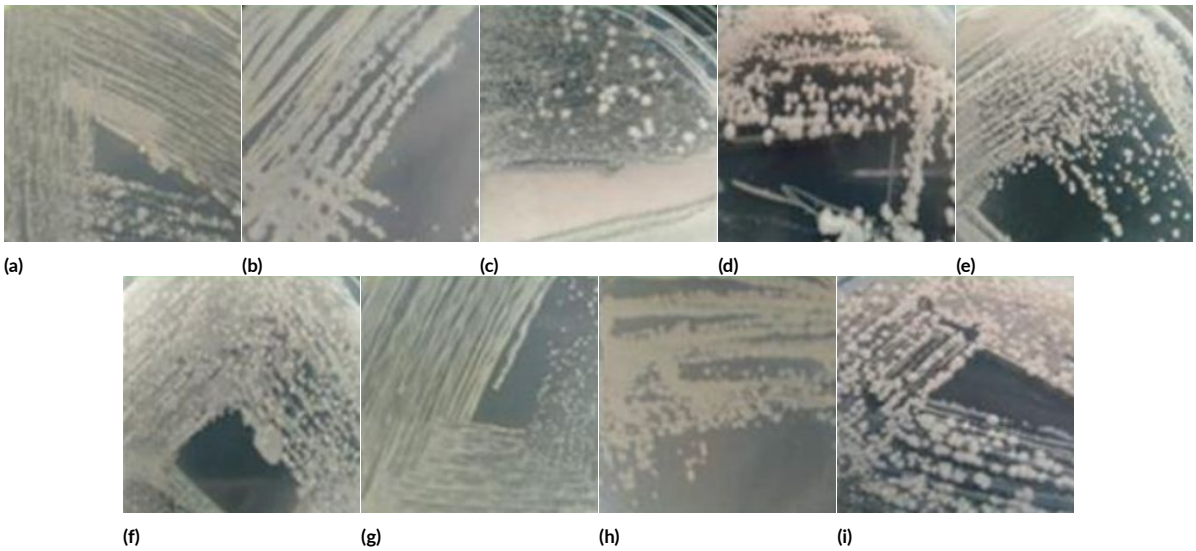



**FIGURE 2** *Jaspis* sp.-associated bacterial strains isolated from Banjar Sari (JABS 1-6) and Bak Blau (JABB 1-3) on SWC agar media after incubating for 48 h at 27 °C. (A) JABS1, (B) JABS2, (C) JABS3, (D) JABS4, (E) JABS5, (F) JABS6, (G) JABB1, (H) JABB2, (I) JABB3

10 mm had moderate inhibitory activity and the diameter of the clear zone <5 mm had weak inhibitory activity.

From overall obtained results, it was noticeable that JABS6 had the best antimicrobial activity against Gram-negative and positive pathogenic bacteria, as well as *C. albicans*, so it was done for further test, which was crude extract assay. Figure 5 displays its result. The diameters of the clear zones measured were 2.8 mm (*E. coli*), 6.2 mm (*P. aeruginosa*), and 2.6 mm (*S. aureus*).

**TABLE 1** Measurement inhibition activity of JABS5 and JABS6 represented by clear zones

| Strains | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | *E. coli* | *P. aeruginosa* | *S. aureus* | *C. albicans* |
| **Bacterial cultures** | | | | |
| JABS5 | 0 | 0 | 6.7 | 0 |
| JABS6 | 24.7 | 8.2 | 0 | 0 |
| **Pellets** | | | | |
| JABS5 | 0 | 4.7 | 3.3 | |
| JABS6 | 0 | 0 | 4.6 | 33.7 |
| **Supernatans** | | | | |
| JABS5 | 0 | 3.9 | 2.6 | 0 |
| JABS6 | 0 | 0 | 3 | 0 |
| **Crude Extracts** | | | | |
| JABS6 | 2.8 | 6.2 | 2.6 | 0 |
| Kontrol Positif* | 14.1 | 19.1 | 15.2 | - |

Note*= 0.005 gr/m of chloramphenicol as a positive control

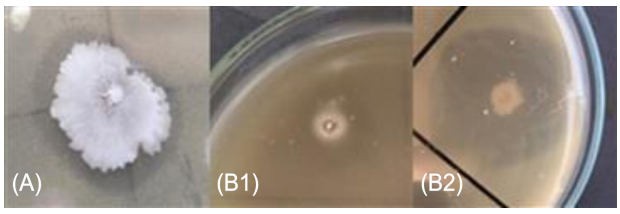



**FIGURE 3** The results of inhibition activity of JABS5 against *S. aureus* (A), JABS6 against *P. aeruginosa* (B1) and *E. coli* (B2)

### 3.3. Potential isolate identification

The PCR amplification resulted in approximately 1284 bp DNA sequences of JABS6 isolate (Figure 6.). Other data also were presented in Table 2, which demonstrated the nearest neighbors of JABS6 registered at the NCBI along with the similarity percentage. The data showed that five species had similar gene sequences to the JABS6 gene. Among these genes, JABS6 had the closest relationship with *Bacillus thuringiensis* strain USS-CAP-1 based on an established phylogenetic tree (Figure 7). Both isolates were genetically similar, with percentages as much as 99.96%.

### 3.4. Discussion

The potential new drugs are more and more needed due to the significant increase of multiresistant microbes. Although the antimicrobial compounds isolated from the terrestrial environment had been analyzed, natural marine compounds from organisms living there displayed consid-

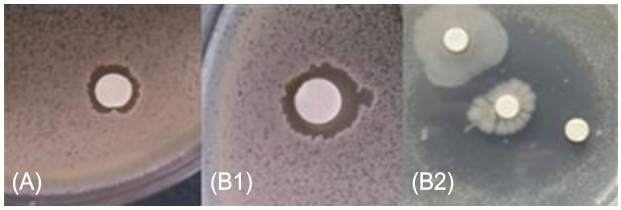



**FIGURE 4** Inhibition zones formed by supernatants of JABS6 against *S. aureus* (A), pellets of JABS6 against *S. aureus* (B1) and *C. albicans* (B2)

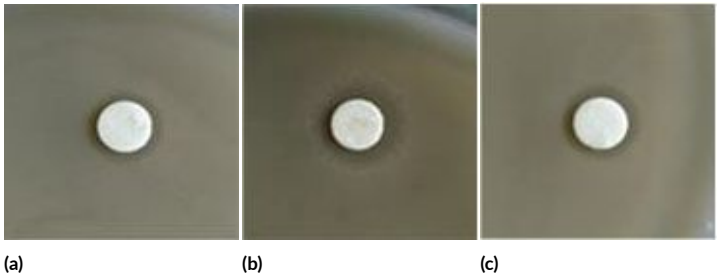



**FIGURE 5** Inhibition zone formed by JABS6 through its crude extracts against *E. coli* (A), *P. aeruginosa* (B), and *S. aureus* (C)

GCTCTCAGAGTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCCATAAGACTGGGATAACTCCGGG
AAACCGGGGCTAATACCGGATAACATTTTGAACTGCATGGTTCGAAATTGAAAGGCGGCTTCGGCTGTCACTT
ATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGTAGCCGACCT
GAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTT
CCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCTGTTG
TTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACT
ACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAG
GTGGTTTCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGT
GCAGAAGAGGAAAGTGGAATTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGAACACCAGTGGCGAA
GGCGACTTTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTA
GTCCACGCCGTAAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGAAGTTAACGCATTAAG
CACTCCGCCTGGGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGA
GCATGTGGTTTAATTCGAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGAAAACCCTAGAGATAG
GGCTTCTCCTTCGGGAGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTA
AGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCATCATTAAGTTGGGCACTCTAAGGTGACTGCCGGTG
ACAAACCGGAAGAAGGTGGGGGATGACGTCAAATCATCATGCCCCTTATGACCTGGGCTACCACGTGCTACAA
TGGACGGTACAAAGAGCTGCAAGACCGCGAGGTGGAGCTAATCTCATAAAACCGTTCTCAGTTCGGATTGTAG

**FIGURE 6** DNA sequences of JABS6 isolate

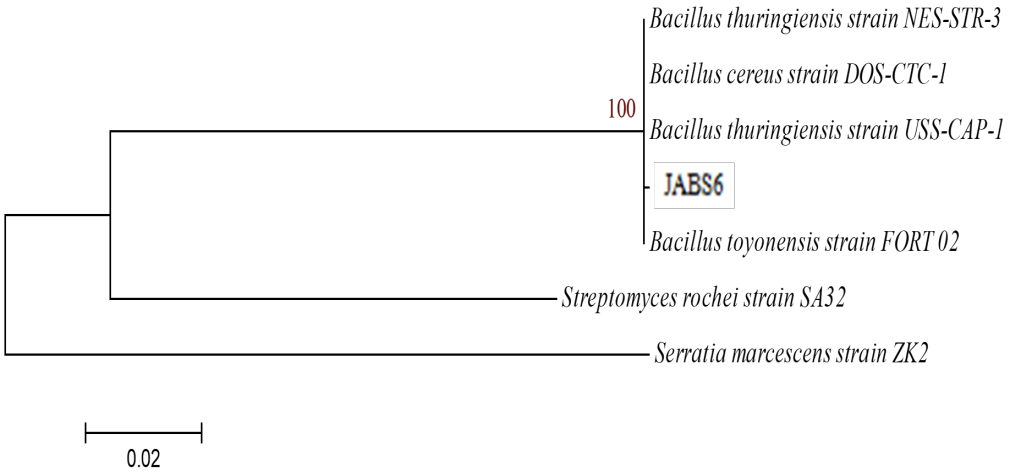



**FIGURE 7** Phylogenetic tree of JABS6 isolate constructed by Neighbor-joining tree approach

**TABLE 2** 16S ribosomal RNA analysis of JABS6 isolate and its similarity (%) to the closest relatives, as well as its accession number

| Isolate | Species Affiliation | Score Total | Query Cover (%) | E-Value | Similarity (%) | Accession Number |
|---|---|---|---|---|---|---|
| JABS6 | *Bacillus thuringiensis* strain USS-CAP-1 | 2350 | 100 | 0.0 | 99.69 | MF083055.1 |
| | *Bacillus cereus* strain DOS-CTC-1 | 2350 | 100 | 0.0 | 99.69 | MF076224.1 |
| | *Bacillus toyonensis* strain FORT 02 | 2350 | 100 | 0.0 | 99.69 | MG561338.1 |
| | *Bacillus mycoides* strain TR 4.1 | 2346 | 100 | 0.0 | 99.61 | MK241977.1 |

erable contributions to be more expanded in the domain of pharmacy (Astuti et al. 2002). Mehbub et al. (2014) suggested that sponges produced the largest amount of biological products worldwide. A total of 2004 components were successfully isolated from marine sponges from 2001 to 2010. The sponges i.e., *Jaspis* sp. utilized in this work, have been known as a source of antimicrobial compounds (Astuti et al. 2002). A total of 774 new compounds were also reported from microorganisms associated with sponges in the last two decades, from 1998 to 2017. These compounds contained various bioactive compounds except for some well-known compounds such as terpenes, alkaloids, peptides, aromatics, meroterpenoids, macrolides, polyketides, steroids, and so on (Cheng et al. 2020).

We reported that 9 bacteria were successfully isolated from *Jaspis* sp. and two of them exhibited antimicrobial activity, i.e., JABS5 and JABS6. Bacterial cultures, pellets, and supernatants assay of JABS5 illustrated the active inhibition towards *S. aureus* and *P. aeruginosa*, even though it was merely weak activity. JABS6, on the other hand, had a growth inhibition on *S. aureus*, *E. coli*, *P. aeruginosa*, and *C. albicans*, which was more potent than JABS5. However, JABS6 isolate was less consistent against Gram-negative bacteria. It was assumed that the cells' structure of Gram-negative bacteria which contain double layer membrane and lipopolysaccharide (LPS) (Snyder and McIntosh 2000) could be an inhibitor to resist hydrophobic and lipophilic molecules. The previous study (Abubakar et al. 2012) unveiled that the bacterial population associated with *Jaspis* sp. from Waigeo island, Indonesia showed antibacterial activity against some multidrug resistance pathogens, including *S. aureus*, *E.coli*, *P. aeruginosa*, and *C. albicans*. Correspondingly, research by Zampella et al. (2000) reported *Chondromyces crocatus* compounds from *Jaspis* sp. which acted as an antimicrobial.

As can be seen from the data of genetic identification, the 16S rRNA gene, it was noticeable that JABS6 isolate was confirmed as *B. thuringiensis* strain USS-CAP-1. This isolate is Gram-positive bacteria, bacil, and is generally distributed in the soil. The colony on the SWC agar media has a pinpoint shape, thick elevation, and entire edge. Although JABS6 isolate also had the same level of similarity with *Bacillus cereus* strain DOS-CTC-1 and *Bacillus toyonensis* strain FORT 02, based on morphological characteristics, physiology, and phylogenetic tree results, JABS6 isolate was confirmed to be closer to *B. thuringiensis* strain USS-CAP- 1 (Table 2). The marine *Bacillus* sp. is discovered in all sponges based on either previous or current studies, such as the study reported by (Pabel et al. 2003). The antimicrobial ability of marine *Bacillus* genus also has been reported by Wahyudi et al. (2018, 2019). They successfully found the inhibition activity from *Bacillus* against *Vibrio* spp., which led to a human illness called vibriosis. Other studies also found the same ability of sponge-associated *Bacillus* collected from the Egyptian Red Sea region (Aboul-Ela et al. 2019), Gujarat Coast of the Indo-Pacific area (Devi et al. 2010), and Algao Bay in South Africa (Matobole et al. 2017). Besides, *B. thuringiensis* has the ability to form crystal proteins during sporulation (Bahagiawati 2002). Bravo et al. (2011) mentioned that *B. thuringiensis* also has insecticidal activity. Briefly, the bacteria strain found is a promising agent for further improvement as antimicrobial and insecticidal.

## 4. Conclusions

This work revealed nine *Jaspis* sp. sponges-associated bacteria collected from the coast of Banjar Sari and Bak Blau, Enggano island. All tests found that JABS6 was the selected strain of its antimicrobial ability and nearly related to *B. thuringiensis* strain USS-CAP-1 based on 16S rRNA analysis. The authors expect this discovery has further potential to be applied in larger fields as an alternative antibiotics.

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## Authors' contributions

S, RH, WD designed the study. RI, UC carried out the laboratory work. S, RH, WD, EN analyzed the data. RI, UC wrote the manuscript. All authors read and approved the final version of the manuscript.

## Competing interests

The author declares that they have no competing interests.

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