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Preliminary Study on Potential Bacterial Species Introducing Antibacterial Agent from Sponge in Unggeh Island Central Tapanuli, Indonesia

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Abstract: Sponges have the potential to symbiont with bacteria that produce antibacterial compounds. This study was conducted to isolate and test the antibacterial activity of sponge symbiont bacteria. Bacterial isolates obtained were tested for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* using the Kirby-Bauer method. The identification results of both sponges have similarities with *Clathria* sp. and *Hyrtios* sp. symbiont bacteria obtained from both sponges as many as 16 isolates. Antibacterial activity testing had a weak inhibitory effect on isolates Sp1A, Sp2C, Sp2D and Sp2F, while Sp2J showed moderate inhibition against gram-negative and positive bacteria. The antibacterial activity of the whole supernatant of symbiont bacteria obtained the diameter of the inhibition zone with weak, medium and strong categories. Sp1A isolate supernatant was obtained, potentially able to fight *Escherichia coli* bacteria with a strong category as a representation of gram-negative bacteria and against gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*) classified into the medium category. In conclusion, 16 symbiont bacterial supernatants have antibacterial activity mostly active against gram-negative bacteria Escherichia coli and gram-positive bacteria Bacillus subtilis. There are 6 isolates (Sp1A, Sp1B, Sp1E, Sp1F, Sp2D, Sp2F) active as antibacterial against *Staphylococcus aureus* with weak and moderate categories.

Keywords: Antibacterial; Sponge; Symbiont bacteria

Introduction

Infectious diseases are a serious problem in the health sector and the biggest cause of death (Cahyani et al., 2019). Infection is a disease caused by microorganisms, one of which is bacteria, most infectious diseases are caused by the bacteria *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. These bacteria can attack all or part of the human body, so antibiotics are needed to prevent and overcome infectious diseases that occur (Darsono & Fajriannor, 2020). The irrational use of antibiotics in overcoming various diseases can cause side effects that cause bacteria to become resistant to certain diseases (Gultom et al., 2021). New and most potential antibacterial compounds are needed in the world of medicine, because some bacteria are already resistant to existing antibacterial compounds (Sunny et al., 2015).

Indonesia is an archipelago that has abundant marine biodiversity, both from plants, animals and microbes. There are many new bioactive compounds in marine organisms, but there is still not much done by researchers in the discovery of potential new antibacterial compounds (Nofiani et al., 2020). One of the

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marine organisms, the sponge, has not been widely explored for its bioactive compounds. Sponges are invertebrate animals that are immobile, soft-bodied (Radjasa et al., 2013) primitive multi-celled animals, unable to move (sessile metazoan) (Cristi et al., 2022).

Sponges have long been recognized as a source of natural products of pharmaceutical and medical relevance (Hentschel et al., 2001). However, obtaining bioactive compounds from marine sources requires a large amount of material. This will damage the marine ecosystem if sponges are exploited continuously. Some studies mention that many bioactive compounds from marine biota are similar to bioactive compounds of microorganisms associated with these marine biota (König et al., 2006). Sponges have a way of eating by filtering seawater (filter feeder) so that in symbiotic relationships sponges accommodate a lot of microorganism diversity (Cristi et al., 2022). There are several types of bacteria that are symbiotic with sponges, due to the influence of the biogeochemical cycle relationship of the main nutrients needed by bacteria symbiotic with sponges, namely carbon, nitrogen and phosphorus nutrient sources (Pita et al., 2018).

Marine microorganisms have extraordinary abilities as producers of secondary metabolites used to fight various diseases (Bibi et al., 2017). Microbes that are symbionts with sponges can produce secondary metabolite compounds, one of which is sponge symbiont bacteria (Cita et al., 2017). Active secondary metabolite compounds from sponges have potential as anti-inflammatory, anticancer, and antimicrobial (Gogineni & Hamann, 2018). Secondary metabolites are natural chemical defences for certain biota that can protect themselves from other living things (Liem et al., 2019). Microbes associated with sponges produce metabolites that are beneficial to invertebrate animals (sponges), these compounds will be absorbed to the surface of their cells or into certain cells for storage and protection of sponges from predators (Salomon et al., 2004). Secondary metabolite compounds extracted from the sponge is the product of microorganisms that are symbiotic with these invertebrate animals (Radjasa et al., 2013). The diversity of microorganisms symbiotic with sponges can produce a source of high-value pharmaceutical products (Gopi et al., 2012). Bioactive compounds in the form of antibacterials produced by bacteria symbiotic with Hyatella sponge produce secondary metabolite compounds that function as antibacterials (Taylor et al., 2007).

The content of bioactive compounds found in sponge symbiont bacteria is still not widely explored, making researchers interested in finding new types of antibacterial-producing bacteria in an effort to reduce antibiotic resistance to pathogenic bacteria. For this reason, it is necessary to conduct research on the antibacterial activity of sponge symbiont bacteria against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. This study can provide information about sponge symbiont bacteria producing antibacterial compounds and can be a reference for developing research on the content of compounds produced by sponge symbiont bacteria in producing new antibacterial compounds.

Method

This research was conducted from June to October 2023. The materials used in this study were bacteria symbionted with sponges from unggeh island, Central Tapanuli. Sponge symbiont bacteria were isolated, antibacterial testing of sponge symbiont bacteria and antibacterial testing of sponge symbiont bacteria supernatant against *Staphylococcus aureus*, *Bacillus subtilis*, and *Eschericia coli*. The method used in this research is experimental. The research data were analyzed descriptively and quantitatively. Sponge sampling was done by exploration method.

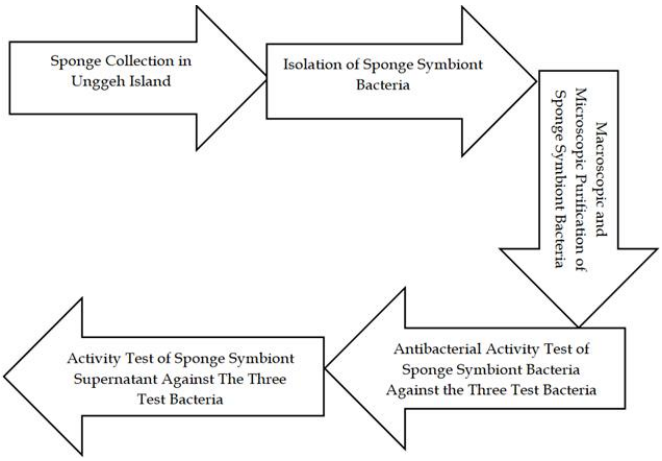


Figure 1. Flow of method stages

Sponge specimens were collected from Unggeh island waters by scuba diving at a depth of 5-8 meters. Sponge specimens were placed into a sterile plastic bag and given oxygen to be brought to the laboratory. Macroscopic identification was carried out to determine the type of sponge obtained. The results of the observations were compared with the guidebook The Sponge Guide (Zea et al., 2014).

Sponge specimens were weighed as much as 1 g, then crushed using a stamper and mortal in sterile conditions (Gopi et al., 2012). The crushed sponge was put into 9 ml of 0.9% NaCl (Nursyam, 2017). The suspension was inoculated into Marine agar media using the pour plate method and incubated at 37 ℃ for 24 hours. Bacteria were purified by looking at morphological characteristics macroscopically and

microscopically. Purification of sponge symbiont bacteria is done by taking a single colony by looking at its morphological characterization by paying attention to size, shape, color, and elevation. The strains obtained were then tested for antibacterial activity.

Antibacterial testing was carried out using the Kirby-Bauer test method using paper discs. The test bacteria *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* were cultured first using Nutrient Agar media. Test bacteria and sponge symbiont bacteria strains were suspended using 0.9% NaCl with Mc farland standard conditions. The test bacteria suspension was taken using a sterile cotton bud and swabbed on the surface of Mueller Hinton Agar media, each sterile disc paper was dripped with 50 µL of sponge symbiont bacteria suspension on the surface of Muller Hinton Agar media, distilled water as a negative control and Ciprofloxacin antibiotic concentration of 200 µg/ml as a positive control, then incubated at 37 ℃ for 24 hours.

Positive results indicate the formation of an inhibition zone diameter around the disc paper. Sponge symbiont bacteria were cultured using Marine Broth media and incubated at 37 ℃ for 24 hours. Cultures were harvested and centrifuged at 6000 rpm for 15 minutes (Sunny et al., 2015). The supernatant obtained was filtered using 0.22 µm sterile syringe filter and tested for antibacterial activity. Each sterile disc paper was dripped with 50 µL of sponge symbiont bacterial supernatant, then the disc paper was placed on Muller Hinton Agar media that had been swabbed with a suspension of test bacteria and positive control using Ciprofloxacin at a concentration of 200 µg/ml. The diameter of the inhibition zone can be categorized as strong for > 20.0 mm in diameter, moderate for > 2.5 - ≤ 20.0 mm and weak for 1.0 mm - ≤ 2.5 mm (Gutiérrez-Barranquero et al., 2019).

Results and Discussion

Based on the results of identification by looking at the morphological characteristics of sponges using The Sponge Guide guidebook. The identification results show that sponge A has similarities with *Clathria* sp. and sponge B has similarities with the *Hyrtios* sp. *Clathria* sp. sponge has the characteristics of a branched growth form - branches, soft and compressible consistency. *Hyrtios* sp. has a bifurcated growth form, if gathered looks black, the surface forms a pyramid, with a skeleton attached in the form of fibers, spicule in the form of a substylostyle. *Clathria* sp. and *Hyrtios* sp. are included in the Demospongiae class (Marzuki & Erniati, 2018).

From both types of sponges successfully isolated and obtained 16 bacterial isolates with each having different characteristics macroscopically and microscopically. *Clathria* sp. like sponge obtained six isolates and *Hyrtios* sp. like sponge amounted to ten isolates.

Table 1. Characteristics of Sponge Symbiont Bacterial Isolates (Sp1) *Clathria* sp.; (Sp2) *Hyrtios* sp.

Isolate	Color	Shape	Margin	Elevation	Gram stain
Sp1A	Clear beige	Circular	Entire	Flat	Bacil (+)
Sp1B	Orange	Circular	Entire	Flat	Bacil (+)
Sp1C	Milky white	Filamentos	Lobate	Flat	Coccus (+)
Sp1D	Beige	Irregular	Curled	Flat	Bacil (+)
Sp1E	Milky white	Irregular	Undulate	Convex	Bacil (-)
Sp1F	Beige	Circular	Entire	Umbonate	Bacil (-)
Sp2A	Beige	Rhizoid	Rhizoid	Raised	Bacil (+)
Sp2B	Clear beige	Irregular	Lobate	Raised	Bacil (+)
Sp2C	Clear beige	Filamentos	Filamentos	Raised	Bacil (+)
Sp2D	Milky white	Circular	Circular	Flat	Bacil (+)
Sp2E	Beige	Irregular	Lobate	Raised	Bacil (+)
Sp2F	Beige	Circular	Entire	Convex	Bacil (+)
Sp2G	Beige	Irregular	Lobate	Flat	Bacil (+)
Sp2H	Milky white	Filamentos	Filamentos	Flat	Bacil (+)
Sp2I	Milky white	Circular	Entire	Flat	Bacil (+)
Sp2J	Milky white	Rhizoid	Rhizoid	Flat	Coccus (+)

Based on Table 1, the six isolates of *Clathria* sp. sponge symbiont bacteria have a dominant circular shape with the dominant color is beige. In isolates of *Hyrtios* sp. sponge symbiont bacteria seen from ten isolates of sponge symbiont bacteria dominated beige with the dominant isolate shape is circular and irregular. Microscopic observations of all isolates of sponge symbiont bacteria are predominantly gram-positive with bacilli cell shape. There are 2 isolates (Sp1C, Sp2J) have a gram-positive coccus cell form. In previous research by Haber et al. (2014), bacteria successfully isolated from the three species of sponges, one of which was dominated by gram-positive. Megawati et al. (2019) stated that as many as 75-85% of marine bacterial cell

forms are bacilli and have flagellates that are used to move actively in the waters while coccus bacterial cells do not have a means of movement so that their life will stick to the substrate including the sponge. Bacteria with coccus cell shape have slimy material so that the cells will bond together to form a solid surface.

(A) (B)

Figure 2. Isolat Sp1F. Notes: A= macroscopic observation; B= microscopic observation

Table 2. Measurement Data Result of Inhibitory Power of Sponge Symbiont Bacteria

Isolate code	*E. coli*	*B. subtilis*	*S. aureus*
Sp1A	-/+	+	-/+
Sp1B	-/+	-/+	-
Sp1C	-	+	-
Sp1D	-/+	-/+	-
Sp1E	-	+	-/+
Sp1F	+	-	-/+
Sp2A	-	+	+
Sp2B	-	+	-/+
Sp2C	+	-/+	+
Sp2D	+	+	-/+
Sp2E	-/+	+	-
Sp2F	+	+	+
Sp2G	+	+	-
Sp2H	-/+	-/+	-
Sp2I	-/+	+	-
Sp2J	+	+	+
Control (+)	++	++	++
Control (-)	-	-	-

The results of the antibacterial activity test in Table 2 show that from all isolates of *Clathria* sp. and *Hyrtios* sp. symbiont bacteria against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, the diameter of the inhibition zone is obtained in the weak and moderate categories. Strains Sp1A, Sp2C, Sp2D and Sp2F had a weak inhibitory effect, while strain Sp2J showed moderate inhibition against gram-negative and positive bacteria. In the research of Gultom et al. (2021) *Clathrina* sp. and *Agelas* sp. sponges from sibolga waters showed that sponge symbiont bacteria (*Bacillus cereus* and *Bacillus paramycoides*) have antibacterial activity against Multi Drug Resistance Organism (MDRO). Research by Rozanah et al. (2023) Sponge symbiont bacteria *Pseudo vibrio* sp. and *Vibrio* sp. isolated from *Phorbas tenacior* can inhibit the growth of pathogenic bacteria *Parahaemolyticus* and *P. atlantica*. In the positive control using ciprofloxacin antibiotics, the diameter of the inhibition zone formed is classified into the strong category. The inhibition formed by the positive control is greater than the inhibition of antibacterial strains.

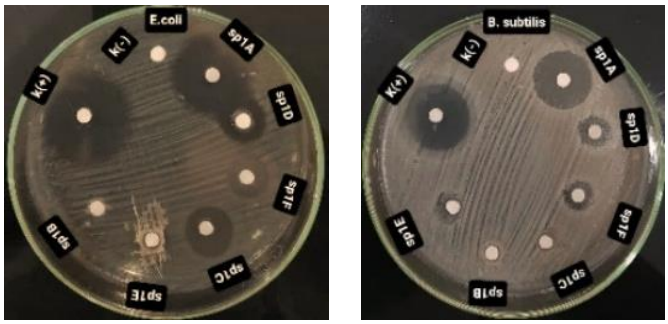
The results showed antibacterial activity of the entire supernatant of the symbiont bacteria *Clathria* sp. and *Hyrtios* sp. obtained inhibition zone diameter with weak, medium and strong categories (Table 3). Of the sixteen isolates showing the greatest antibacterial activity was found in the supernatant of strain Sp1A, the potential inhibition was proven to be able to fight *Escherichia coli* bacteria with a strong category as a representation of gram-negative bacteria and against gram-positive bacteria (*Bacillus subtilis* and *staphylococcus aureus*) classified into the medium category.

Table 3. Measurement Data Results of Inhibition Power of Sponge Symbiont Bacterial Supernatant

Isolate code	*E. coli*	*B. subtilis*	*S. aureus*
Sp1A	++	+	+
Sp1B	-/+	-/+	-/+
Sp1C	+	+	-
Sp1D	+	+	-
Sp1E	-/+	+	-/+
Sp1F	+	+	+
Sp2A	+	+	-
Sp2B	-/+	-	-
Sp2C	-	-	-
Sp2D	-/+	-/+	-/+
Sp2E	-/+	-	-
Sp2F	+	-	+
Sp2G	+	+	-
Sp2H	-	-	-
Sp2I	-	+	-
Sp2J	-	-	-
Control (+)	++	++	++
Control (-)	-	-	-

The strength of the supernatant inhibition of strain Sp1A against gram-negative bacteria is stronger than gram-positive, the diameter of the gram-negative inhibition zone formed has the same category as the inhibition of ciprofloxacin positive control. This indicates that the antibacterial power of Sp1A supernatant is as strong as that of the positive control in inhibiting *E. coli* bacteria. In the research of Cita et al. (2017), the symbiont bacteria *Xestospongia testudinaria* had stronger antibacterial activity against gram-negative bacteria (*E. coli* and *Klebsiella pneumoniae*) compared to gram-positive bacteria (*Bacillus subtilis*). Supported by research by Judianti et al. (2014) stated that bacteria symbiotic with sponges *Spongia* sp. and

Hippospongia sp. have the ability to inhibit the growth of *E. coli* and *S. aureus* bacteria. In another study conducted by Amraoui et al. (2016), showed that the symbiont bacteria sponge *Ircinia spinulosa* (Sarcotragus) has a broad spectrum in inhibiting pathogenic bacteria *E. coli*, *P. fluorecens*, *Pseudomonas sp*., *Bacillus* sp., *S. aureus* and *E. faecalis*.



(A) (B)

Figure 3. Observation of supernatant activity of sponge symbiont bacteria. Notes: A = against the test bacteria Escherichia coli; B = against the test bacteria Bacillus subtilis

The results showed that the antibacterial activity of the supernatants was mostly active against gram-negative bacteria and only three bacterial supernatants (Sp2H, Sp2I, Sp2J) had no antibacterial activity against *E. coli* bacteria (Table 3). This allows the presence of secondary metabolite compounds in the active supernatant as antibacterial. Secondary metabolites in the form of renieramycin, an alkaloid class active from symbiont bacteria *Haliclona* sp. have potential as antimicrobials (Gogineni & Hamann, 2018). Secondary metabolites in the form of alkaloids can have potential in the pharmaceutical world. Alkaloids are a very potential source of medicinal materials because they have considerable therapeutic abilities (Rumalolas et al., 2023). As much as 35-60% of the total mass of the sponge in the mesohyl there are microorganisms associated with the sponge (Taylor et al., 2007). Microbes that are symbionts with sponges will produce chemical compounds as a form of defense response for their hosts (Tianero et al., 2019). Bacteria symbiotic with sponges isolated from *Hyrtios* sp. which are included in the strains of *Pseudomonas aeruginosa* SNP0614, *Staphylococcus sciuri* DSM 20 345 and *Alcaligenes faecalis* strain NBRC 13111 have inhibitory effects as antibacterial against *E. coli* and *V. parahaemolyticus* (Rini et al., 2017). Research by Trianto et al. (2019) showed that sponge symbiont bacteria isolated from the Demospongie class are *Bacillus clausi*, *Virgibacillus chiguensis*, *Bacillus tropicus*, *Paracoccus marcusii*, *Bacillus tropicus*, *Vibrio parahaemolyticus*, *Bacillus paramycoides*, *Virgibacillus dokdonensis*, *Virgibacillus dokdonensis*, *Virgibacillus dokdonensis* produced secondary metabolites that were active as antibacterial against *Staphylococcus aureus* and *Bacillus subtilis*.

Conclusion

Based on the results of the study, isolates of *Clathria* sp. and *Hyrtios* sp. like sponge symbiont bacteria have antibacterial activity from supernatants mostly active against gram-negative bacteria *Eschericia coli* and gram-positive bacteria *Bacillus subtilis*. There were isolates (Sp1A, Sp1B, Sp1E, Sp1F, Sp2D, Sp2F) active as antibacterial against *Staphylococcus aureus* with moderate and strong inhibitory activity.

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Author Contributions

Contributed in conducting research, analyzing data, and writing articles, L.N.A; became a supervisor in research activities and writing articles D.S, K.N, J.M.

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Conflicts of interest

The authors declare no conflict of interest.

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