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Review Article

A Mini Review on Analysis of Potential Antibacterial Activity of Symbiotic Bacteria from Indonesian Freshwater Sponge: An Unexplored and A Hidden Potency

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1 ABSTRACT

Marine sponges have been investigated as potential bioresources because of their symbiotic relationship with microbes such as Actinobacteria that produce antibacterial substances. In contrast, a group of sponges, that inhabits freshwater environments called freshwater sponges (Order Spongillida Manconi & Pronzato, 2002) and consists of only one percent among all of the sponges' species (Phylum Porifera Grant, 1836), has not yet intensively examined. For this reason, we screened, determined, evaluated, and reviewed by examining several databases in Scopus, Pub Med, and Google Scholar related to potential aspects of symbiotic bacteria and their antibacterial substances that can be further utilised and developed into synthesised antibacterial compounds, based on published metagenomic data of symbiotic bacteria in freshwater sponges. At the same time, we compared a composition of those freshwater symbionts to marine sponges' symbionts whether those possess a similar composition or not. Moreover, a current report and a revisit study of freshwater sponges in East Java, initiate further direction on mapping of those symbiotic bacteria from Indonesia that can be nominated as potential groups possessing antibacterial properties.

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INTRODUCTION

Sponges (Phylum Porifera Grant, 1836) are multicellular animal that has been known for hosting various microbes e.g., bacteria, archaea, fungi, or microalgae organisms as symbionts and comprises 40 % of sponges' volume furthermore, symbionts might possess a unique and specific relationship with sponges' host (Hentschel et al. 2003; Fieseler et al. 2004; Webster & Taylor 2012). Besides providing energy for a certain host sponge, e.g., photosynthetic cyanobacteria, other microorganisms contribute to

sponges' defense mechanism through the production of a bioactive compound or secondary metabolites (Unson et al. 1994; Schmidt et al. 2000), which has been researched intensively for a decade as antibacterial potency of bacterial symbionts from sponges. In Indonesia, several bio-prospecting studies have been conducted on screening these bacteria symbionts in sponges for antibacterial potency, particularly from marine sponges e.g., *Xestospongia testudinaria* from Papua (Cita et al. 2017), *Aaptos suberitoides*, *Agelas nakamurai* and other eight marine species from Sulawesi (Riyanti et al. 2020), *Spongia officinalis* from Nusa Tenggara (Prastiyanto et al. 2022) and *Jaspis* sp. from Enggano (Sipriyadi et al. 2022).

In contrast to marine sponges, freshwater sponges (Manconi & Pronzato 2002) are classified as a minor group of sponges (Phylum Porifera Grant 1836) that inhabit freshwater environments and ecosystems such as lakes and rivers. These sponges consist of only 1% of all sponge species, which contains around 8000 species according to the World Porifera Database (WPD), (de Voogd et al. 2023). A prominent feature of freshwater is the ability to survive in a fluctuating or extreme environmental condition like a shortage of water and the ability to individual dispersion over a long distance because of possessing an asexual reproduction organ called gemmules (Manconi & Pronzato 2002). Ecologically, freshwater sponges are linking energetic pathways between the pelagic and benthic community in the freshwater ecosystem by hosting zoochlorella and being eaten by spongivorous insects (Skelton & Strand 2013). Besides being recognised as a maritime country, Indonesia also possesses an abundance of freshwater streams (approximately 170 main rivers), with the longest river (Kapuas River) measuring up to 1143 km and a basin area of 98.740 km² (Suwarno et al. 2013). However, despite possessing a lot of freshwater streams, data on the diversity of freshwater sponges from Indonesia is overlooked.

As marine sponge symbiont often plays an important role in bioactive compounds production such as a member of Actinomycete, Proteobacteria, and firmicutes phyla, it is expected that sponge symbionts of freshwater sponges are members of these phyla (Bibi et al. 2017). Therefore, this mini review on comparing the composition of bacterial symbionts in a different environment can be used for exploring and mapping a potential sponge symbiont from a freshwater environment, especially Indonesian freshwater sponges, which can be utilised as an alternative source of antibacterial substances.

DISCOVERED “ANTIBACTERIAL-LIKE SUBSTANCES” FROM SPONGES

Joseph¹ et al. (2017) reported the antibacterial activity of *Streptomyces pharmamarensis* isolated from the marine sponge *Clathria procera*. This symbiont exhibits antibacterial activity against *Acinetobacter baumannii*, *Enterococcus faecalis*, and even resistant strains such as methicillin-resistant *Staphylococcus aureus*. Based on TLC analysis, the antibacterial activity was due to two¹⁶ unknown substances recognised as PVI401 and PVI402. Furthermore, HR-LC-MS (High-Resolution Liquid Chromatography and Mass Spectroscopy) devices confirmed PVI401 and PVI402 are substances that structurally like phosmidosine and altermicidine, despite FTIR (Fourier-transform infrared spectroscopy analysis) device reported a functional group of substances did not match with the functional group of phosmidosine and altermicidine. Therefore, PVI401 and PVI402 possibly are novel compounds.

An antibacterial activity of Symbiotic bacteria isolated from a ma-

rine sponge *Haliclona* sp. was reported by Asagabaldan et al. (2017). This species is known as a source of antibacterial substances since the sponge extract contains Haliclolin A. However, symbionts of *Haliclona* sp. might also harbor and involve antibacterial properties. In this study, one of the isolates, PSP39.04 isolate, also exhibited antibacterial activity against *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *A. baumannii*, and *S. aureus*. PSP39.04 was closely related to *Chromohalobacter salexigens* by 16S rRNA. For this reason, it is deduced antibacterial activity of PSP39.04 was due to its ability to produce cyclic peptides, which are effective against Multi-Drug Resistant (MDR) pathogens. Moreover, it is concluded while sponge extracts may exhibit bioactive properties, antibacterial compounds derived from symbionts are more effective since their activity against drug-resistant strains and pathogens commonly found causing diseases in humans (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. abbreviated as ESKAPE). In addition, because marine sponge symbionts possess potent antibacterial properties, symbionts of freshwater sponges might potentially exhibit a similar activity.

COMMUNITY STRUCTURE AND DIVERSITY OF BACTERIA SYMBIONT IN MARINE SPONGES RELATES TO ANTIBACTERIAL PRODUCTIONS

Altuğ et al. (2021) reported two different structures of bacterial communities from two different sponges collected from the Aegean and Marmara seas. Sponges collected from the Aegean seas were consisted of *Sarcotragus* sp., *Cocopongia scalaris*, *Acinella cannabina*, *Ircinia* sp. *Chondrosia reniformis*, *Agelas oroides*, *Sarcotragus spinosulus*, *Scalariispongia scalaris*, *Crambe crambe*, *Chondrosia reniformis*, *Aplysina aerophoba*, *Petrocia fisciformis*, while sponges collected from the sea of Marmara were consisted of *Ciocalypta penicillus*, *Ficulina ficus*, *Dictyonella plicata*, *Haliclona mediterranea*, *Rapailia* sp., and *Hymeniacion perlevis*. The study reported that phylum Proteobacteria (synonym: Pseudomonadota) was a notable microbe symbiont and frequently found among sponge samples from both Aegean and Marmara seas with percentages of 86% and 82% respectively. Furthermore, the percentage of phylum Proteobacteria in both seas consists of 30% of class Alphaproteobacteria, 8% of Betaproteobacteria, and 48% of Gammaproteobacteria from the Aegean Sea while the sample from the Marmara Sea only consists of two classes of Proteobacteria, which are 27% Alphaproteobacteria and 55 % of Gammaproteobacteria only. Furthermore, 8% and 18% of class Flavobacteria from phylum Bacteroidota were also recorded. Concurrent with that 6% of the Bacilli class from phylum Bacillota was solely recorded from The Aegean Sea. While the sponge samples show a high abundance of proteobacteria, the water samples show a moderate level of proteobacteria. In this study, Altuğ et al. (2021) also measured the antibacterial activity of crude sponges' extract. The result showed that the methanol extract of the sponges collected from the Aegean Sea showed stronger antibacterial activity than the samples collected from the sea of Marmara. Therefore, they suggest different environmental conditions obviously effects on antibacterial activity of the sponge and its bacterial symbiont.

A subsequent study by Pires et al. (2020) in Asian waters reported a community structure of bacterial symbionts isolated from several sponges identified as *Aaptos lobata*, *Xestospongia testudinaria*, *Stylissa carteri*, and *Stylissa massa* at Tioman Islands, Malaysia. Phylum Cyanobacteria was recorded as the most abundant symbiont in the *S. carteri*, while Phylum Chloroflexi (synonymized as Chloroflexeota) currently was

abundant in *X. testudinaria* (39%) followed by *A. lobata* (16%). This study also stated that Chloroflexi were commonly found in High Microbial Abundance (HMA) sponges, as it carries out an important role in sponges' diet in converting inorganic to organic carbon. Furthermore, Phylum Actinobacteria (synonymized as Actinomycetota currently) was found to be highly abundant in the *A. lobata* (27%) and *X. testudinaria* (17%). Actinobacteria are prolific antibacterial producers and play an important role in the host's defense mechanisms.

A following study by Retnowati et al. (2021) recorded the community structure of *Callyspongia* sp. from Kepulauan Seribu, Jakarta, Indonesia. *Callyspongia* sp. is a marine sponge species that belong to the order Haplosclerida Topsent, 1928 where a group of freshwater sponges (suborder Spongillina Manconi & Pronzato, 2002, currently invalid suborder rank) was used to be a member of the order Haplosclerida before elevated into order Spongillida Manconi & Pronzato, 2002 that is exclusively group for sponges inhabit the freshwater environment. The metagenomic data shows symbiont of *Callyspongia* sp is consisted of seven phyla with the highest abundance of 82 % Proteobacteria (currently synonymized as Pseudomonadota), followed by 12 % of Acidobacteriota, 2 % of Planctomycetota, 2% of Actinobacteria (currently synonymized as Actinomycetota), 1.08 % of Bacteroidetes (currently synonymized as Bacteroidota), 0.61 % of Firmicutes (currently synonymized as Bacillota), and 0.09% of Cyanobacteria.

Furthermore, the dominant abundance of Proteobacteria was noted and mentioned as a common occurrence in Low Microbial Abundance (LMA) Sponges (Giles et al. 2013). Moreover, among those studies on the Aegean Sea, Marmara Sea, Tioman Island Malaysia, and Kepulauan Seribu, Jakarta Indonesia shows that Proteobacteria are one of the most important phyla of marine sponges' symbionts, corroborate Proteobacteria as symbionts carry out and possess various crucial functions such as nitrogen fixation, host defense mechanism, and nitrification process (Mohamed et al. 2010). Likewise, the transcriptomic data from Moitinho-Silva et al. (2016) revealed that one genome bins namely "Cc Phy" from the family Phyllobacteriaceae from Proteobacteria involve metabolic production, synthesized between sponge *Cymbastela concentrica* –microbe symbiosis, besides other genome bins 'CcThau' and "CcNi" produced by the sponge and genus Nitrospira of bacterium and order Nitrospumillales of thaumarchaeal respectively.

COMMUNITY STRUCTURE AND DIVERSITY OF BACTERIA SYMBIONT IN FRESHWATER SPONGES

In contrast to symbionts of marine sponges, the community structure of bacterial symbionts in the freshwater sponge is overlooked. For this reason, it results in a lack of information on "antibiotic-like substances" discovered from bacterial symbionts of freshwater sponges. An example of metagenomic data of *Ephydatia fluviatilis*, a cosmopolite freshwater sponge from the Netherlands (Costa et al. 2013), shows some bacterial phyla TM7 and BLUT were absent in the water. But it was found in the sponge with a substantial proportion of 22% and 9.1% respectively. It was reported that sequences from the Chlamydia phylum were present in a low abundance of 1.5% in the sponge but absent in the water. Furthermore, some noticeable differences in the abundance of certain phyla were also recorded that a higher abundance of Actinobacteria (40.9%) and Bacteroidetes (15.4%) than in the sponge sample (12.1% and 9.8% respectively). Together, despite the similar abundance of Proteobacteria phylum in the sponge and water, the domination of class Betaproteobacteria (71%)

and low abundance of Alpha- (24.4%) and Gamma- (2.25%) Proteobacteria were different. It happened because a proportion of Alpha-, Beta-, and Gamma- Proteobacteria were rather equal in a sponge (40%, 22.5%, and 37.5% respectively). Moreover, in the Bacteroidetes phylum, class Sphingobacteria accounts for 100% of the Bacteroidetes in the sponge whereas, Flavobacterium of Bacteroidetes dominated water (65%). The unusual existence of Phylum Chlamydia symbiont in freshwater sponges is comparable to marine sponges for antibacterial properties since Chlamydia mostly possesses a pathogenic character. Dharamshi et al. (2022) discovered two new Families: Candidatus Sororchlamydiaceae fam. Nov and Candidatus Parasimkaniaceae fam. Nov in three marine sponges' species *Haliclona* spp., which presumably possess novel natural products.

Graffius et al. (2023) discovered 380 isolates of symbiotic bacteria from freshwater sponges *Spongilla lacustris* that were sampled in Austria and consisted of 197 cultured vs 183 uncultured bacteria. Furthermore, among the 197 cultured symbionts, 33 isolates that represented 31 bacteria genera e.g., *Micrococcus* sp., *Streptomyces* sp., *Ensifer* sp., *Roseateles* sp., *Rhizobium* sp., and *Masilia* sp., possess secondary metabolite biosynthesis gene clusters (BGCs). Moreover, the four highest number BGC per genome are two *Streptomyces* isolates SL203 and SL294, *Bacillus* sp. SL112 and *Gordonia* sp. SL206 respectively, which reveals those bacteria possess a potential producer of metabolites, which have a wider range of biological effects, including antibacterial, antifungal, and cytotoxic activities.

Clark et al. (2022) isolated 522 symbionts from two specimens of freshwater sponges *Eunapius fragilis* that were sampled from St Lawrence River USA. They utilised another technology which is called Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and discovered at least four phyla commonly associated with sponges: Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes. Among those four phyla, 11 genera e.g., *Paenibacillus*, *Streptomyces*, *Bacillus*, *Micromonospora*, and *Pseudomonas*, are reported for having potential as bioactive producers, known as Specialized Metabolites (SMs) group.

Sugden et al. (2022) were investigating the microbiome composition of freshwater sponges *Ephydatia muelleri* from three different locations in Canada. They discovered that the composition of sponges' symbionts is Proteobacteria, Bacteroidetes Actinobacteria, Cyanobacteria, Planctomycetes, and Verrucomicrobia. However, the most important finding is, the composition of symbionts among three specimens in all locations are significantly different, which is obviously influenced by geographical location and habitat.

Investigation on Actinobacteria reported that order Acidimicrobiales has become the most common symbionts in *E. fluviatilis* that might possess antibiotic and cytotoxic properties (Keller-Costa et al. 2014). This order also possesses the ability to oxidize or reduce iron and oxidize sulfur. The data also shows a common ribotype of *Pseudomonas* shared across four *E. fluviatilis* specimens. *Pseudomonas* has previously been reported for their ability to produce secondary metabolites, therefore further study regarding the ribotype may result in a better understanding of the ecological role of *Pseudomonas* in this symbiotic relationship.

Laport et al. (2019) showed the metagenomic data of other species of freshwater sponge *Tubella variabilis* from Pernambuco, Brazil. The study reported a higher OTU richness in sponges (3762 – 4709 OTUs) than in surrounding waters (3419 – 3522). The majority of discovered phyla were Proteobacteria, Verrucomicrobia, and Cyanobacteria, whereas 39 phyla and candidate phyla were reported as a minor abundance. Fur-

thermore, Proteobacteria were discovered in great abundance in both sponges (60 – 82%) and water (85-89%). Despite a high abundance of Proteobacteria, dissimilarity among classes between sponge and water was recorded as Betaproteobacteria was dominant in the sponge, while water was dominated by Alphaproteobacteria. At the same time, a significant difference was also observed for phylum Bacteroidetes, in which abundance in sponges was significantly higher compared to water samples. Furthermore, class Cytophagia, which is a member of Bacteroidetes was reported to be a hundred times higher in sponge water samples.

Seo et al. (2016) recorded the community structure of three freshwater sponge species in Lake Baikal, Russia, *Lubomirska baicalensis*, *Baikalospongia intermedia*, and *Swartschewskia papyracea*. Six bacterial phyla can be found among the samples, which were Cyanobacteria, Proteobacteria, Actinobacteria, Bacteroidetes, Planctomycetes, and Verrucomicrobia. Cyanobacteria were found to be the most abundant in all sponges with the highest value found in *B. intermedia* (78%), *L. baicalensis* (70%), and *S. papyracea* (43%). Furthermore, Cyanobacteria discovered in those three samples were dominated by the genus *Prochlorococcus*. However, it should be noted that *Prochlorococcus marinus* can only be found in *S. papyracea* while symbiont from phylum actinobacteria was also found in a higher abundance in *S. papyracea*.

Kumar et al. (2020) reported a diversity of microbe symbionts on freshwater sponge *Spongilla* sp. and marine sponges *Ciocalypta* sp. from a wetland ecosystem in Gujarat, India. They recorded bacterial symbionts from various phyla such as Actinobacteria, Firmicutes, Chloroflexi, Planctomycetes, Acidobacteria, and Gemmatimonodetes as well as a few candidates' phyla such as BRC1, GN02, GN04, H-178, KSB3, NKB19, OD1, OP8, SR1, TM6, TM7, WPS2, WS1, WS3, WS4, WWE1, and ZB31. Proteobacteria were recorded as the most abundant phyla in both sponges despite different compositions for marine sponges *Ciocalypta* sp. was dominated by Alpha-, Beta-, and Gammaproteobacteria class, while in freshwater sponges *Spongilla* sp. Delta-, and Epsilonproteobacteria were the dominant class.

In another study by Gaikwad et al. (2016) in India, metagenomic data of freshwater sponge *Eunapius carteri* and *Corvospongilla lapidosa* symbionts in lake Talegaon Dabhade and Pashan respectively were discovered. The study reported a difference in community structure in sponge and water, in which sponge samples possess the highest abundance in the Firmicutes phylum and are followed by proteobacteria. Furthermore, the highest abundance of OTU belonged to the genus *Clostridium* (50.3%) followed by *Synechococcus* (8.67%). At the same time, there are also OTUs that are exclusively found in sponges such as *Acinetobacter*, *Vogesella*, and *Rhizobiales*. Those reported data in contrast to the community structure of water because, the highest OTU symbiont in a sponge, genus *Clostridium* is absent in water, which the disparity of abundance infers heritability factor in symbiont's related to hosting specificity and community structure. The capability of *Clostridium* to utilize a sponge extracellular matrix that is rich in glycoproteins, proteoglycans, spongin, and other organic substances might also allow the sponge to achieve a high abundance of OTU. Moreover, some members of the *Clostridium* are known to be able to do fermentation and exhibit antibacterial activity when cultured with *Pseudomonas* and *E. coli*.

HOST SPECIFICITY AND SYMBIONT RELATIONSHIP ON SYNTHESISING ANTIBACTERIAL PROPERTIES

Once discussing sponge symbionts, host specificity plays a huge role in

the community structure of symbionts in Figure 1 (Carrier et al. 2022). It is important that symbionts are a genealogical factor in sponges. Therefore, the sponge may have a unique community structure of symbiont, i.e., lateral gene transfers of symbionts living in mesohyl occur through a pathway when the sponge embryo is brooded or developed externally. Furthermore, oocytes of sponges obtain symbionts by directly engulfing them or through the help of nurse cells. When the nurse cells acquire symbionts, they transfer symbionts via a cytoplasmic bridge or phagocytosed by the oocyte. Alternatively, the brooded embryos may obtain symbionts from two pathways, infiltration of the follicle or nurse cells containing symbionts through cleavage furrows, or direct infiltration of the symbiont through the space between follicles. Moreover, while recruiting symbiotic bacteria from ambient water, epithelial cells of the sponge assist free-living bacteria from surroundings water to adhere to its surface and enter sponge mesohyl forming a pocket-like structure.

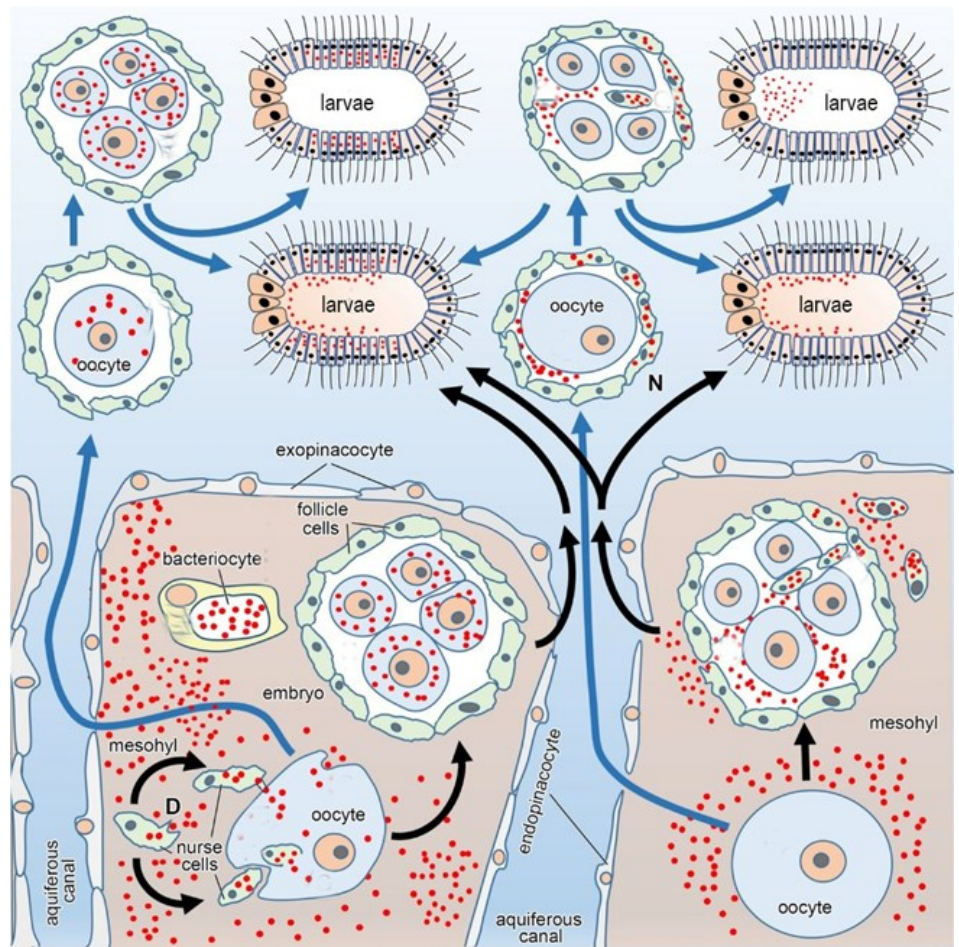


Figure 1. A modified schematic diagram from the study and permission of copyright from Carrier et al. (2022) explains two pathways of symbionts through vertical transfer, which make sponges possess a unique community structure of bacterial symbiont from the surrounding water. Sponge mesohyl's or bacteriocytes were inhabited freely by microbes (red dots) occur transmitted from mother to offspring (black arrows) or develop externally (blue arrows).

Host specificity of symbionts is a crucial factor in linking sponge symbiont-derived compounds productions. It specifically refers to which sponge should discover for obtaining a certain symbiont. Sponge symbiont has been previously explored for various properties as they have exhibited. A study by Sirpu Natesh et al. (2018) shows the anticancer properties of *Bacillus subtilis* isolated from the marine sponge *Clathria frondifera* through activation of the caspase-3 protein in the human breast can-

cer cell line. Another study by Schmidt et al. (2000) recorded the antifungal activity of the symbionts from the marine sponge *Theonela swinhoei*. These symbionts contain a novel peptide, recognised as “Theopalauamide” and have been proposed to be classified as *Entotheonella palauensis* as a subdivision of Gammaproteobacteria. Furthermore, Kaluzhnaya et al. (2012) recorded the activity of symbionts from the freshwater sponge *Lubomirskia baicalensis* in producing Non-Ribosomal Peptide Synthase (NRPS) and Polyketide Synthase (PKS). Moreover, it was recorded that symbionts from the phylum Cyanobacteria produce PKS I, while symbionts from the phyla Proteobacteria and Cyanobacteria produce NRPS/PKS hybrid. The abundance of bioactive compounds should be considered since sponge symbionts have exhibited antibacterial and antifungal properties i.e., productions of antibacterial compounds commonly utilize bacteria from Actinobacteria phylum, as well as bacteria from Genus Bacillus and Pseudomonas because those bacteria are exhibited antibacterial properties (Dita et al. 2017; Lee 2020)

POTENCY OF BACTERIAL SYMBIONTS FROM FRESHWATER SPONGES IN INDONESIA

While marine sponge has been studied extensively regarding the community structure of the symbionts and the antibacterial compounds produced by the symbionts, the study of freshwater sponge symbionts either regarding the community structure or the antibacterial compounds producing is overlooked. Therefore, only a minor data on the metagenomic study of freshwater sponge microbiome or symbiotic microbe and a lack of data from freshwater sponges in Indonesia. For this reason, the first step is performing metagenomic analysis or symbionts profiling of the identified freshwater sponges from Indonesia. Currently, The WPD listed 14 species of freshwater sponges that are recorded in Indonesia (Manconi et al. 2013; de Voogd et al. 2023). Two species from families Metaniidae Volkmer-Ribeiro, 1986; *Metania pottsi* (Weltner, 1895), *Metania vesparium* (Martens, 1868) recorded in Borneo, followed by 11 species of Family Spongillidae Gray, 1867. Those are *Stratospongilla sumatrana* (Weber, 1890) that are recorded from Sumatra Island, *Ephydatia fortis* Weltner, 1895, *Ephydatia ramsayi* (Haswell, 1883) *Radiospongilla cerebellata* (Bowerbank, 1863), *Radiospongilla crateriformis* (Potts, 1882), *Radiospongilla indica* (Annandale, 1907), *Trochospongilla latouchiana* Annandale, 1907 *Umborotula bogorensis* (Weber, 1890), *Eunapius carteri* (Bowerbank, 1863) recorded Java Province, *Nudospongilla vasta* (Weltner, 1901) from Sulawesi, and *Rosulaspongilla alba* (Carter, 1849) from Bali. Another two species from two different families were additionally added currently, which are *Pachydictyum globosum* (Weltner, 1901) recorded in Sulawesi (Meixner et al. 2007) and *Oncosclera asiatica* Manconi & Ruengsawang, 2012 recorded in Java, Figure 2 (Setiawan et al. 2023) from the family Malawispongiidae Manconi & Pronzato, 2002 and Potamolepidae Brien, 1967 respectively.

Furthermore, there is few culturable sponges' symbionts and can be processed by a culture-dependent test to screen the symbionts. Finally, we should identify the antibacterial compounds from the culture in which this step is applicable for sponge study in general. Many of the recorded symbionts that previously exhibited antibacterial activity were only reported as cell extract or culture extract. Simple productions by fermentation are the first cornerstone in the sponge-symbiont-derived technology. For the next step, it could be multiple routes that can be explored. The first route is the co-culture route, as some symbionts could have a

higher yield of antibacterial compounds. Kanagasabhapathy and Nagata S (2008) reported a higher antibacterial activity of epibiotic bacteria isolated from marine sponges *Pseudoceratina purpurea* when cross-cultured with pathogenic bacteria *Staphylococcus aureus* and *Bacillus licheniformis*. It was deduced that the higher antibacterial activity is induced by quorum sensing as chemical signals for competitive bacteria resulting in higher production of antibacterial substances.

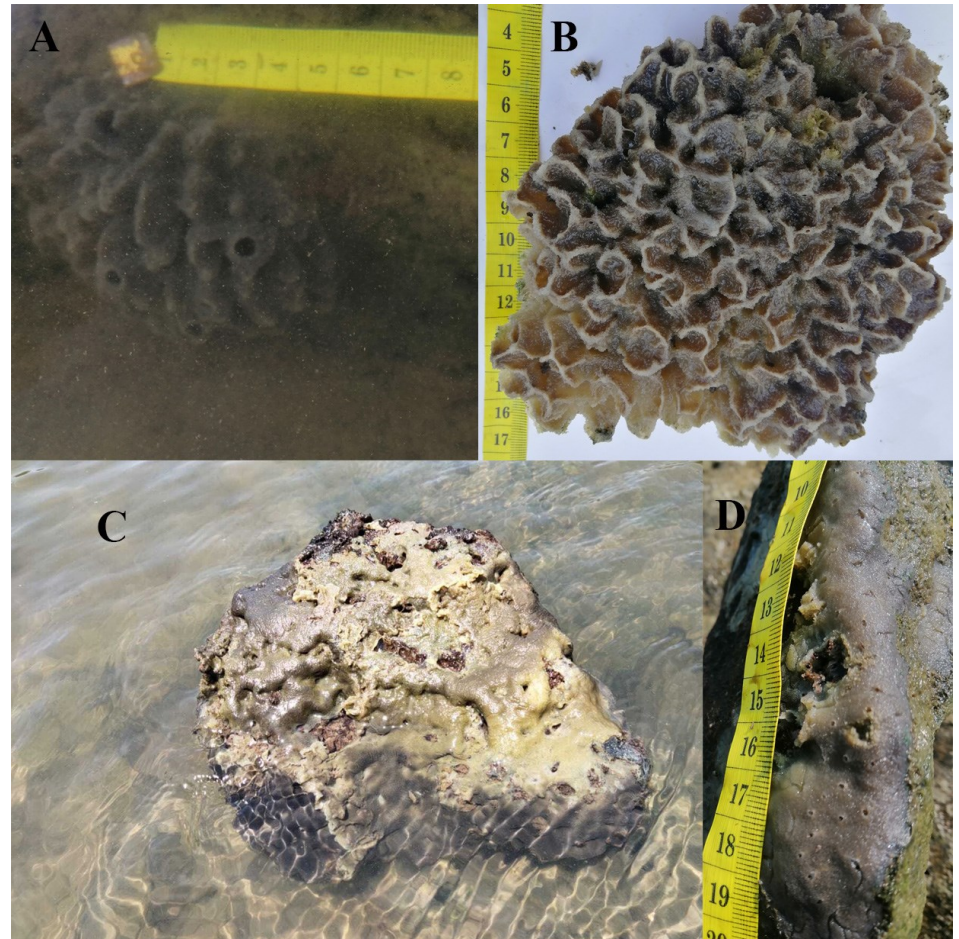


Figure 2. A & B., Specimen of *Eunapius carteri*, a cosmopolite freshwater sponges' species, and C & D, *Oncosclera asiatica*, which currently reported inhabiting part of Porong river, East Java, Indonesia (Setiawan et al. 2023)

An antagonistic assay is a further approach besides the cross-culture method where instead of a cooperative relationship between one symbiont and another, a competitive relationship between symbionts is detected. This process aims to screen symbionts with the highest antibacterial activity, which can be used as a donor for genetic modification. An example of this process can be observed in a study by Riyanti et al. (2020) (Figure 3). They reported symbionts from 10 species of marine sponges from Sangihe islands, North Sulawesi, Indonesia, for antibacterial activity screening. Symbionts of these samples are selected through a few screenings for antibacterial activity. In this step, isolated symbionts that consisted of 12 genera were present, which covers two third of the strains belonged to *Bacillus* (66.7%), followed by *Pseudomonas* (6.5%), *Staphylococcus* (5.6%), *Lysinibacillus* (4.6%), and *Solwaspora* (3.7%), were cross cultured with two pathogen species of bacteria: the gram-negative *E. coli*, and the gram-positive *Micrococcus luteus*. After 48 hours of incubation, those identified and isolated symbionts show a clear zone, and furthermore determined as “active” produce antibacterial activity. Among 835 isolates, only 108 isolates (12%) exhibited antibacterial activity. Further-

Table 1. Sponge symbionts and their antibacterial compounds.

Bacterial Symbiont	Phylum	Host sponge	Habitat	Produced Antibacterial Compound	Note	References
<i>Micrococcus luteus</i>	Actinomycetota	<i>Xestospongia</i> sp.	Marine	Lutoside	Exhibits antibacterial activity to <i>Staphylococcus aureus</i> , <i>Vibrio anguillarum</i> , and <i>Candida albicans</i>	(Bultel-Poncé et al. 1998)
<i>Brevibacterium</i> sp.		<i>Calyspongia</i> sp.	Marine	Phenazine-carboxamide 1,6-phenazine-dimethanol	Exhibits activity against <i>Enterococcus hirae</i> and <i>Micrococcus luteus</i>	(Choi et al. 2009)
<i>Streptomyces</i> sp. Actinomycetota		<i>Baikalo-spongia bacillifera</i>	Fresh water	Variipeptin	Exhibits antibacterial activity against <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , and <i>Candida albicans</i>	(Axenov-Gribanov et al. 2016)
<i>Pseudonocardia</i> sp.				Culture extracts	Exhibits antibacterial activity against <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , and <i>Staphylococcus carnosus</i>	
<i>Janibacter limnosus</i>		<i>Lubomirska bai-calensis</i>	Fresh water	Helquinoline	Exhibits activity against <i>Streptomyces viridochromogenes</i> , <i>Staphylococcus aureus</i> , <i>Mucor miehei</i> , <i>Chlorella vulgaris</i> , <i>Chlorella sorokiniana</i> , and <i>Scenedesmus subspicatus</i>	(Kaluzhnaya et al. 2021)
<i>Rathayibacter</i> sp.				Tunica-minyluracil	Exhibits potent activity against gram-positive bacteria, especially species of <i>Bacillus</i> .	
<i>Janthinobacterium</i> sp.	Pseudomonadota	<i>Lubomirska bai-calensis</i>	Fresh water	Violacein	Exhibits activity against gram-positive bacteria increases cell permeability followed by rupture of cytoplasmic membrane	(Belikov et al. 2021)
<i>Bacillus</i> sp	Bacillota	<i>Metania reticulata</i>	Fresh water	Cyclosporin A	Exhibits activity against gram-positive bacteria <i>Staphylococcus aureus</i> and fungus <i>Aspergillus</i> sp.	(Rozas et al. 2015)

more, among 108 isolates, only 4.6% show activity against *E. coli*, 78.7% show activity against *M. luteus*, and 16.7% show activity against both *E. coli* and *M. luteus*. Despite combination competition assay revealed the highest antibacterial activity (*Bacillus* sp.), producing compounds like surfactin. However, this method is only applicable for culturable bacteria.

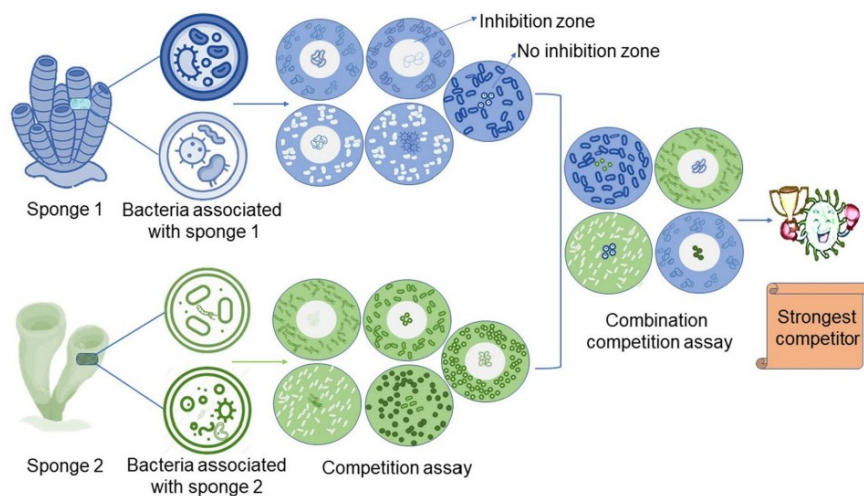


Figure 3. The diagram of the methodology from the permitted copyright of Riyanti et al. (2020) is to cross-culture symbionts and to determine the highest activity and most potent antibacterial property.

A further alternative is utilising genetic modification tools. After the identification of an antibacterial substance. We could observe which proteins have roles for producing the potential antibacterial compound. The gene of interest could select and insert into a vector. Furthermore, the inserted vector is transformed into host *E. coli*. Moreover, it would increase the production of antibacterial substances.

POTENTIAL SYMBIONTS OF BACTERIA

Actinobacteria: a diverse multipotential symbiont

Actinobacteria are one of the most known phyla for their ability to produce bioactive compounds. The compounds produced by bacteria have been recorded as possessing antibacterial, antifungal, antiparasitic, anti-malarial, immunomodulatory, antioxidant, and even anticancer properties (Mayer & Hamann 2005; Bull & Stach 2007; Pimentel-Elardo et al. 2010; Blunt et al. 2018). Compounds produced by actinobacteria include polyketides, alkaloids, fatty acids, peptides, and terpenes. Several investigations on sponge-derived actinobacterial products i.e., the antibacterial activity of sponge actinobacterial symbiont from either marine or freshwater environments can be listed in the following Table 1. Two examples of marine sponges, *Xestospongia* and *Callyspongia* have been recorded to host actinobacteria with antibacterial properties. *Xestospongia* and *Callyspongia* were members of the order Haplosclerida, where freshwater sponges were previously assigned to a sub-order Spongillina (disused and unaccepted rank currently, see World Porifera Database in Manconi & Pronzato 2002) as a member of order Haplosclerida. Currently, freshwater sponges have been established and elevated as ranked into order Spongillida (Morrow & Cárdenas 2015; de Voogd et al. 2023) despite the fact that Spongillida and Haplosclerida are phylogenetically shared a closely related Actinobacteria – derived antibacterial compounds, in which those compounds have been recorded to be highly effective against gram-positive bacteria (Cartwright et al. 2020). At the same

time, almost every compound presented in freshwater sponges in the Table 1 has exhibited a potential activity against *Staphylococcus* bacteria. The potential antibacterial activity against gram-positive pathogenic bacteria also exhibits the importance of biofilm prevention. Conjugation in gram-positive bacteria requires cell-to-cell contact. This conjugation commonly transfers resistant genes, therefore by preventing cell-to-cell contact, bacteria susceptibility to antibacterial compounds is conserved.

In freshwater sponges, two examples from endemic species of Baikal Lake Russia, have been explored on having symbiotic microbe possessing antibacterial potency. First, isolates of *Streptomyces* sp. from *Baikalospongia bacilifera* is producing Variapeptin, which is like the antibacterial activity of Azinotricin family and shown to inhibit the growth of numerous gram-positive bacteria, but not gram-negative or fungus (Axenov-Gribanov et al. 2016). Second, three bacterial symbionts of another freshwater sponges from Baikal Lake Russia, *Lubomirska baicalensis* are reported to have the ability to produce secondary metabolites that are important for antibacterial compounds (Kaluzhnaya et al. 2021). Those compounds are Helquinoline which exhibits high antibacterial and antifungal activity from the *Janibacter limosus*. Furthermore, *Rathayibacter* on producing Tunicaminyuracil antibiotics.

Pseudomonas: the most ubiquitous symbiont

Pseudomonas is a genus of the proteobacteria phylum (Gammaproteobacteria) that can be found in as symbionts in marine and freshwater sponges with a range of moderate to high abundance and possess a potential producer as a source of antibacterial compounds. A study by Keller-Costa et al. (2014) on freshwater sponge *Ephydatia fluviatilis* recorded the antibacterial activity of various *Pseudomonas* symbionts against common human pathogens such as *Bacillus subtilis*, which also reported showing anti-biofilm properties. *Pseudomonas* can produce compounds in the phenazine group. The metabolism of phenazine is regulated by the *Phz* gene cluster (*Phz A, B, C, D, E, F, G*). Depending on which gene takes part in the regulation, there are four main products of phenazine metabolism: Phenazine (PHZ), Pyocyanin (PYO), Phenazine-1-carboxamide (PCN), and Phenazine-1,6-dicarboxylic acid (PCD). The production of phenazine is affected by extracellular signals, as for symbiotic *Pseudomonas*, these extracellular signals are a means of communication with the sponge host. The signals are received by the GacA/ GacS protein complex which would start a cascade reaction with the Rsm protein group (Bilal et al. 2017). The method of action of the Phenazine compound group is to induce a cellular redox reaction. When Phenazine is introduced to the cell, it would penetrate the cell wall and infiltrate the mitochondria. In the mitochondria, Phenazine would act as an electron acceptor, forming the O²⁻ and ONOO⁻ superoxide. These oxides are highly radical and may damage the cell's organelles (Briard et al. 2015). Moreover, another compound called Violacein isolated from symbiotic bacteria *Janthinobacterium sp* is reported to be an important antibacterial compound. This symbiont is also isolated from freshwater sponges of Baikal Lake Russia, *Lubomirska baicalensis* (Kaluzhnaya et al. 2021).

Bacillus: biosurfactant and its broad applications

Bacillus is one of the most versatile bacteria in biosynthesis since it can produce a broad variety of bioactive compounds such as non-ribosomal peptide synthase (NRPS) and Polyketide synthase (PKS) (Prastiyanto et al. 2022). Furthermore, bacillus could also produce compounds with anti-

bacterial properties. According to Rozas et al. (2015) reported the anti-bacterial activity of *Bacillus* sp. isolated from the Amazonian freshwater sponge *Metania reticulata*. The culture extract exhibits antibacterial activity against *Staphylococcus aureus*, after mass spectroscopy and HPLC analysis, it is concluded that the compounds producing this bactericidal effect were similar in structure to Cyclosporin A. Cyclosporine is produced by Cyclosporin synthase (CySyn). It is made from D-alanine, (4R)-4-[(E)-2-butyl]-4-methyl-L-threonine (Bmt), and L-2-aminobutyric acid) (see detail Lawen 2015). First D-alanine is activated, then delineated from the sequence of the *sim A* gene. This delineation opens an open reading frame that would be translated into Cyclosporine A. Cyclosporine A anti-bacterial activity was due to its ability to inhibit cell wall synthesis. Cyclosporine would bind with cyclophilin creating a protein complex, this complex would then bind with Calcineurin which prevents dephosphorylation of the lipid carrier in the cell wall synthesis (Masaki & Shimada 2022).

CONCLUSIONS

The diversity study of freshwater sponges in Indonesia is overlooked, which made a lack of data on symbiotic bacteria from the region. Nevertheless, symbionts' potency that possesses antibacterial properties can be predicted and mapped by comparing the composition of the sponges that are recorded in Indonesia to similar species in another region e.g., *Eunapius carteri* and *Spongilla* sp., from India. Currently, studies on symbionts of freshwater sponges are mapping the diversity, and groups of Actinomycete, Proteobacteria, and Firmicutes, have been recognised as possessing potential antibacterial compounds. Moreover, identification, followed by synthesising antibacterial compounds produced by Indonesian freshwater sponges is needed, since most of described antibacterial compounds e.g., Helquinoline and Violacein are mostly described from Russian freshwater area at Baikal Lake. At the same time, differences in geographical habitat despite similar freshwater sponges' species influence the discrepancy of symbionts' composition, and obviously, a bioactive compound produced by the symbionts.

AUTHOR CONTRIBUTION

M.E, ES & A.B designed research on reviewing literature process, analysed data and wrote manuscripts. N.A, E.P, C.R, D.W, suggested on improvement on every important aspect related on manuscript.

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CONFLICT OF INTEREST

There is no conflict of interest among authors on this mini-review paper.

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