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# Antibacterial potential of nudibranch-associated bacteria from Saparua and Nusa Laut Islands, Indonesia

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**Abstract**. *Kristiana R, Sibero MT, Farisa MY, Ayuningrum D, Dirgantara D, Hanafi M, Radjasa OK, Sabdono A, Trianto A. 2019. Antibacterial potential of nudibranch-associated bacteria from Saparua and Nusa Laut Islands, Indonesia. Biodiversitas 20: 1811-1819.* Infections caused by multidrug-resistant bacteria are the international health issue that triggers the urgency of finding new antibacterial agents. The aim of this study was to obtain the nudibranchs-associated bacteria that have bioactivity against multidrug-resistant bacteria. A total of 13 species of nudibranch were identified based on morphological characterization. Overlay methods were used for the screening of the isolates bioactivity against six pathogenic multidrug-resistant bacteria. The Minimum Inhibition Concentration (MIC) of the crude extract was evaluated against Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, *Bacillus subtilis*, *Klebsiella pneumonia*, *Micrococcus luteus*, and Extended Spectrum Beta Lactamases *E. coli* (ESBL) using MTT method. A total of 145 isolates were obtained which eleven of the isolates showed antibacterial activity against the pathogenic bacteria. The MIC tests showed that the best activity was isolate SM-S-9-15 and SM-N-3-7. The methanolic extract of isolate SM-S-9-15 active to all of the pathogenic bacteria, while the ethyl acetate extract of the isolate SM-N-3-7 active to the *E. coli*, *B. subtilis*, *K. pneumonia* at the concentration of 500 µg/mL. According to 16S ribosomal RNA gene sequence-based identification, all active isolates belong to *Virgibacillus marismortui*, *V. dokdonensis*, *Bacillus kochii*, *Vibrio algynoliticus*, and *Pseudoalteromonas piscicida*.

**Keywords:** Nudibranch, associated-bacteria, *Multi-drug Resistant*, antibacterial activity

## INTRODUCTION

The rise in the prevalence of multidrug-resistant (MDR) human pathogens has increased the fatal incidence to at least 700,000 deaths annually worldwide (Wang et al. 2019). A pathogenic bacteria may be categorized as MDR if the pathogen is insusceptible to at least one agent in three or more antibiotic classes (Magiorakos et al. 2012; Basak et al. 2016). Several pathogens such as MRSA, *E. coli*, *B. subtilis*, *K. pneumonia*, and *M. luteus* have been reported as MDR pathogens. Of these, *B. subtilis* and *M. luteus* are commonly isolated as nosocomial bacteria, as they cause infections in the hospital (Panghal et al. 2015; Banawas et al. 2018). Between 2001 and 2016, the worldwide incidence of MRSA infections has increased by more than 10% that has led to the increased difficulty of treatment with currently marketed drugs (Lee et al. 2018). Additionally, Scheuerman et al. (2018) reported that ESBL and *K. pneumoniae* cause high mortality rates in patients with a bloodstream infection. The high number of MDR infections and the few choices in antibiotics to treat them indicates the urgency for exploration of new antibiotic candidates.

Marine invertebrates have been considered as the prospective sources of bioactive compounds (Senthilkumar and Kim 2013; Miller et al. 2018; Vlachou et al. 2018). Among all marine invertebrates, sponges are widely accepted as being the most prolific source of bioactive compounds. Compared to sponges, the Indonesian nudibranch is much less explored, especially concerning bioactive compounds. Nudibranchs, or marine sea slugs, are marine invertebrates that are classified as phylum Mollusca, classed as Gastropods, ordo Nudibranchia (Harris 1973; Arbi 2011; Chavanich et al. 2013). Nudibranch can easily be recognized in nature with its morphological characteristic, namely the slug-like form, absence of an outer shell and the presence of rhinophores that function as its eyes (Arbi 2011). However, several novel compounds have been reported as having been isolated from nudibranch. Namely the 4-isocyano-9-amorphene and 10-isocyano-4-amorphene from *Phyllidiella pustulosa*, *P. ocellate* (Sim et al. 2018), dendrodoristerol from *Dendrodoris fumata* (Huong et al. 2019) and finally xidaoisocyanate A and bisformamidokalihinol A from *Phyllidiella* sp. that feed on a marine sponge *Acanthella cavernosa* (Wu et al. 2019).

However, large quantities of nudibranch are required for the production of the bioactive compounds, and direct extraction from nature could be unsustainable for the ecosystem in regards to the function of nudibranch in the marine food web (Ramirez et al. 2017; Cunha et al. 2018; Davis et al. 2018). Therefore, a study of nudibranch-associated bacteria as a source of bioactive compounds is strongly proposed to find their bioactivity against MDR. A study by Calvacanti et al. (2008) reported nudibranch *Tambja eliora* produces alkaloid tambjamine D as cytotoxic and genotoxic compounds. Riyanti et al. (2009) reported having successfully isolated 27 associated bacteria from *Jorunna* sp. and *Chromodoris* sp. Moreover, Böhringer et al. (2017) studied the diversity of nudibranch-associated bacteria from North Sulawesi, Indonesia and the potential of the bacterial isolates as a source of antimicrobial substances which resulted in 35 of 49 isolates with antimicrobial activity dominated by the genus *Pseudoalteromonas* and *Vibrio*. Our report aims to isolate the nudibranch-associated bacteria with antibacterial potential against MDR bacteria.

## MATERIALS AND METHODS

### Sample collection sites

Nudibranchs were taken in September 2018 at four sites of Maluku Province, Indonesia (Figure 1): Site 1 ($128^0$ 33' 9.3" E, $3^0$ 29' 97.3" S/Saparua Island); Site 2 ($128^0$ 42' 7.38" E, $3^0$ 25' 77" S/Saparua Island); Site 3 ($128^0$ 47' 97.6" E, $3^0$ 38' 37.1" S/Nusa Laut Island); Site 4 ($128^0$ 48' 70.9" S, $3^0$ 38' 74.7 E"/Nusa Laut Island). Specimens were stored in zip-lock plastic bags at depth 5-30 m by scuba diving. Each specimen was collected according to scientific needed for conservation consideration (Marine regulations 2016). All collected specimens were kept in a cool-box for the source of associated bacteria (Hooper 2003, Kang et al. 2013). Underwater photography of each specimen was taken during diving for documentation. The specimens were chosen based on the morphological appearance according to identification book of nudibranch.

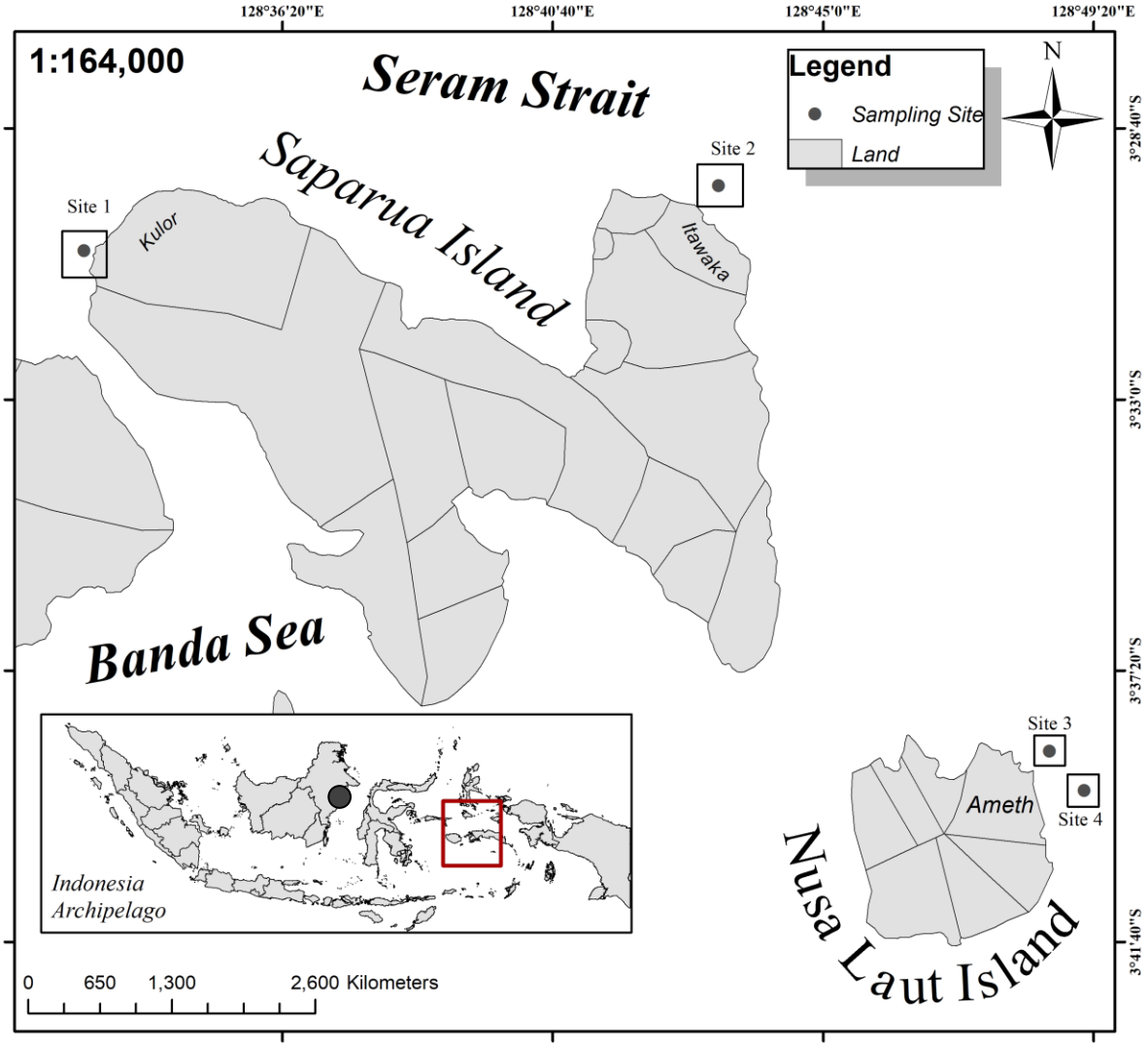


**Figure 1.** Sampling site in East Indonesian Archipelago. Redline is indicated area sampling. Black circles are indicated specific location. Site one and two are in Saparua Island and site three and four are in Nusa Laut Island, Maluku, Indonesia

**Isolation of nudibranch-associated bacteria**

Specimens were rinsed using sterilized seawater (SSW) to remove impurities. Each specimen was separated into two sections, namely the body and viscera parts, and subsequently smoothly crushed with a sterile mortar and pestle. Bacterial isolation was performed using a dilution method (Gupta et al. 2017). This was done serially from $10^{-1}$, $10^{-3}$ and $10^{-4}$. Diluted nudibranch fragments were plated on Nutrient Agar (NA) (Oxoid UK). The composition of the NA was as follows: Lab-Lemco $6.10^{-5}$ g/L, Yeast Extract $1{,}3.10^{-5}$ g/L, Peptone $3.10^{-4}$ g/L, Sodium Chloride $3.10^{-4}$ g/L and Agar $1.10^{-3}$ g/L. The inoculation with bacteria was done with the spread method and incubation lasted for 24 hours at 32°C. Based on the morphological characteristics, bacterial colonies were separated and purified in sequence onto fresh NA media in order to obtain a pure culture (Ramos 2004; Risan 2017).

**Screening of antibacterial activity**

Using the overlay method, the bioactivity of the isolates was screened in the following order against MRSA, *E. coli*, *B. subtilis*, *K. pneumonia*, *M. luteus*, and ESBL. The isolates were then inoculated onto NA media using a streak method and incubated for 24 hours stored at 32°C. The MDR bacteria were cultivated in a ten mL Nutrient Broth (NB) medium. The composition of the NB was 1 g/L Lab-Lemco powder, 2 g/L yeast, 5 g/L peptone, and 5 g/L sodium chloride, in 1 liter of SSW. Cultured MDR was then placed in an orbital shaker for 24 hours. The density of the cultured MDR was measured with a 0.5 McFarland standard. The isolates in the NA soft media was mixed with 1% of the inoculated MDR and incubated for 24 hours at 37°C (Muhialdin et al. 2012).

**Mass cultured and extraction of crude extract**

The bacterial isolates that showed potential were cultivated in a liquid medium to stimulate the production of antimicrobial compounds. The fresh colony was inoculated in 25 mL of Zobell broth medium (peptone and yeast) as the starter of mass culture. The isolate was cultured in a 250 mL flask. The mass cultures were transferred from 250 mL flasks to one liter Erlenmeyer flasks and incubated in a shaking incubator at 110 rpm and 37°C for four days (Radjasa and Sabdono 2003).

Harvesting was done by 15 minutes of centrifugation of cultured bacteria at 8,000 rpm and at 4°C. The pellet of bacteria was extracted with methanol (MeOH) and the medium was extracted in a separation funnel with ethyl acetate (EtOAc) at a ratio of 2: 1 between solvent and medium, respectively. The solvents (MeOH and EtOAc) were evaporated in a rotary evaporator and the mass of each crude extract was calculated. The obtained crude extract was further tested for antibacterial activity.

**Antibacterial activity of crude extract**

The bacterial extract was tested against all of six MDR bacteria using 96 well plates with suspended bacteria. The 96 well plates were incubated overnight at 37 $^0$C in a shaker at 210 rpm and using MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) methods as color-based indicators of antibacterial activity. Vancomycin was used as positive control and DMSO (Dimethyl sulfoxide) were used as a negative control (Ayuningrum et al. 2019).

**16S rRNA gene sequence-based identification of active bacteria of potential isolates**

Identification of potential isolates that have an active response against the MDR pathogens was done using molecular identification. The DNA extraction was carried out using the Chelex method (Walsh et al. 1991) with several modifications that are explained by Sibero et al. (2019a). Selected colonies were inoculated in 50-100 µL $ddH_2O$ and 1 mL of 0.5% saponin in a phosphate buffer solution (PBS) 1× (stored overnight). The mixture was centrifuged at 12,000 rpm for 10 minutes (1 rpm = 1/60 Hz), after which the supernatant was discarded. Then, 100 µL $ddH_2O$ and 50 µL of 20% Chelex 100 was added to the final solution and boiled for ten minutes and vortexed once for five minutes. The mixture was centrifuged at 12,000 rpm for ten minutes and stored at -20°C. The bacterial DNA that was used for 16S rRNA gene sequencing was amplified with a polymerase chain reaction (PCR) using the universal primers 27F (5′AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′TACGGTTAACCTTG TTACGACTT-3′). The PCR conditions used in this study were in accordance with Ayuningrum et al. (2017), namely as follows. Initial denaturation at 95°C for three minutes followed by 30 cycles of denaturation at 95°C for one minute per cycle, annealing at 53.9°C for one minute, extension at 72°C for one minute, and the final extension at 72°C for seven minutes. The next step was gel electrophoresis with a voltage of 100 volts for a duration of 30 minutes. The visualization of the PCR product was done using UVIDoc HD5 (UVITEC Cambridge, UK). The amplified isolate was then processed through a DNA sequencer in Genetica Science, Jakarta.

The sequenced isolates were aligned using the MEGA 7.0.26 software package followed by BLAST (Basic Local Alignment Search Tool) analysis to discover bacterial similarities. All aligned nucleotide sequences of potentially anti-pathogenic bacteria have been deposit in the GenBank National Center for Biotechnology Information (NCBI) database under assigned accession numbers shown in Table 2. Phylogenetic tree analyses were conducted with the neighborhood-joining (NJ) tree method described in Kristiana et al (2017). This way, the diversity of anti-pathogenic bacteria associated with nudibranch was investigated.

## RESULTS AND DISCUSSION

**Identification of nudibranch**

Nudibranch can be recognized easily in nature with the morphological characteristic i.e. the slug like-form, absence of outer shell and the presence of rhinophore that has vision function (Arbi 2011). Table 1 and Figure 2. shows the result of nudibranch identification. Numerous studies in Indonesia reported on the diversity of

nudibranchs from several locations. Bunaken National Park in North Sulawesi is one location that well surveyed. Four surveys by Eisenbarth et al. 2018 documented more than 200 species, which approximate 50% of the specimens have been identified, and the rest still unknown. In this study, a total of 13 specimens were collected and identified based on the morphological characteristics according to identification book "Nudibranch Behavior" (Behrens et al. 2005).

Table 1 shows the 13 nudibranchs that were collected from the Saparua and Nusa Laut Islands. The nudibranchs were identified as nine different species based on their morphological characteristics. Behrens et al. (2005) and Coleman (2001) stated that morphological identification is commonly applied to identify the nudibranch until the species level because every species has a specific color pattern and formation. *C. lochi* was the most abundant species at the sampling sites. The several specimens belonging to *C. lochi* were 18-SM-N-3, 18-SM-N-6, 18-SM-N-7, 18-SM-N-10, and 18-SM-S-7. Specimen 18-SM-N-14 is suggested as *C. annae* because of its blue with darker markings and lack of a mid-dorsal longitudinal line; small black specks within the blue areas (Eisenbarth et al. 2018). At this location, three different species of genus *Phyllidiopsis* were collected and identified as *P. pipeki* (18-SM-N-2), *P. fissurata* (18-SM-N-8), and *P. krempfi* (18-SM-S-11). Pavlov and Britayev (2012) stated that the notum of *Phyllidiopsis* is relatively broad, hard, usually covered by a few short tubercles towards the notal edge and several large conspicuous tubercles medially. The rhinophores are lamellate and the rhinophoral pockets are well defined contractile sheaths with smooth edges. The rudimentary gill cavity encircles the anus (Martynov et al. 2012).

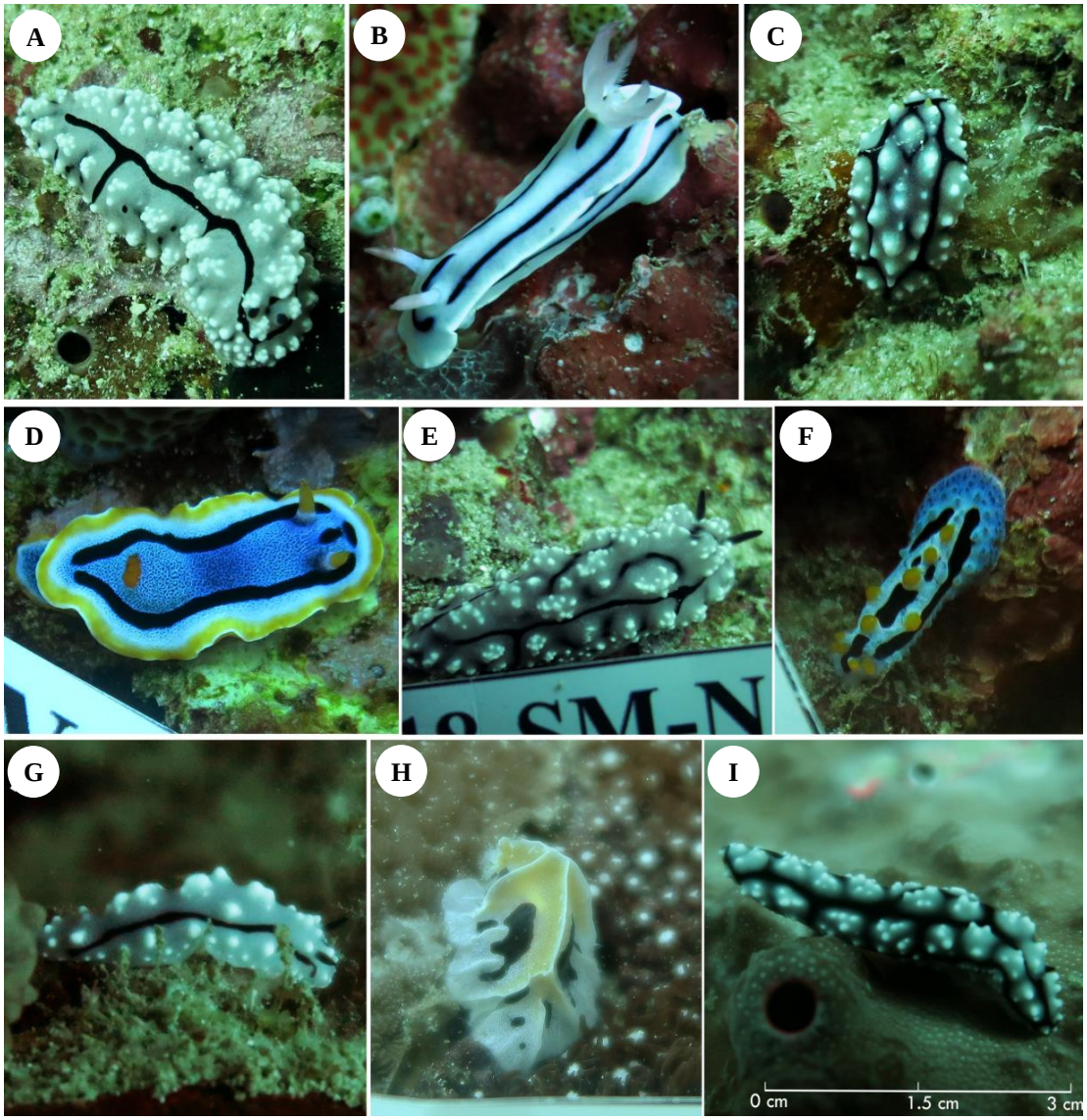


**Figure 2**. Nudibranchs collected from Saparua and Nusa Laut Islands, Maluku, Indonesia. Note: A. *Phyllidiopsis pipeki*; B. *Chromodoris lochi*; C. *Phyllidiopsis fissurata*; D. *Chromodoris annae*; E. *Phyllidiella pustulosa*; F. *Phyllidia coelestis*; G. *Phyllidiopsis krempfi*; H. *Reticulidia fungia*; I. *Phyllidiella cooraburrama*

Sample 18-SM-N-2 had two longitudinal black lines that are only present in *P. pipeki*. The presence of the moderately broad and hard notum helps to identify the N-8 as *P. fissurata*, as well as the two main black lines that run laterally and are connected through the middle part of the notum by a few transversal lines. The rhinophores are pinkish anteriorly and black posteriorly. The specific shape and color pattern of *P. krempfi* existed in sample 18-SM-S-11. The two specimens 18-SM-N-19 and 18-SM-S-17 are identified as *P. pustulosa* and *P. cooraburrama*, respectively. The identification of 18-SM-N-19 as *P. pustulosa* was determined by the presence of predominantly black with pink or white tubercles. Specimen 18-SM-S-17 has characteristic pinkish with a network of dorsal black lines is also visible on *P. cooraburrama*. Specimen 18-SM-N-22 belongs to *Phyllidia coelestis* with the specific characteristics such as blue with black color between the ridges, yellow tubercles, and rhinophores. Furthermore, 18-SM-S-9 was assigned as *Reticulidia fungia* which has specific characteristics such as the bluish-white mantel with a few broad, smooth, orange ridges and white crests.

The diversity of nudibranch is influenced by food, habitat, coral coverage, evolution, and environment (Korshunova et al. 2017). Generally, nudibranchs eat algae, sponges, hard and soft corals, bryozoa and hydroids (Allen and Steene, 1999). Saparua and Nusa Laut Island are noted as having environmental condition such as temperatures, height waves, foods and substrates that support the diversity of this invertebrate (Mulyadi 2011). Moreover, the presence of sponge in the environment suggests a dominance of *C. lochi* in the sampling sites.

**Isolation and screening antibacterial activity**

From the collected nudibranchs specimens, a total of 145 bacterial isolates were obtained. The isolates were tested against six MDR pathogenic bacteria that resulted in 11 active isolates as shown in Table 2.

**Table 1.** Identification of the collected nudibranch from Saparua Island, Maluku Province, Indonesia

| Sample ID | Proposed species | Key identification* |
|---|---|---|
| 18-SM-N-2 | *Phyllidiopsis pipeki* | The body color is grey with a pinkish hue and two longitudinal black lines; some specimens with a few black spots or several black rays extending to the edge of the mantle; tubercles large, single or compound; rhinophores black and pink, with a black line posteriorly. Size: 4 cm. Habitat: on the reef flats in 10-20 m depth |
| 18-SM-N-3<br>18-SM-N-6<br>18-SM-N-7<br>18-SM-N-10<br>18-SM-S-7 | *Chromodoris lochi* | The body color is pale blue with a dark blue submarginal band and mid-dorsal line; rhinophores and gill range from yellow to pink. Size: 3.5 cm. Habitat: on the walls of the fringing and barriers reefs where it feeds on sponges |
| 18-SM-N-8 | *Phyllidiopsis fissurata* | Grey with tall, multi-compound pink tubercles; dark grey to black pigment forming lines and deep fissures between the tubercles; rhinophores pink with black posteriorly. On sharp drop-offs and walls. Size: 4.5 cm |
| 18-SM-N-14 | *Chromodoris annae* | Blue with darker markings and lacking a mid-dorsal longitudinal line; small black specks within the blue areas. On open rock walls and reef faces where it feeds on aplysillid spinges in 15-30 m. Size: 4 cm |
| 18-SM-N-19 | *Phyllidiella pustulosa* | Highly variable in color pattern, predominantly black with pink or white tubercles; tubercles arrange in small clusters, becoming more separated as the animal grows. In shallow water reefs to deep reef slopes, 5-40 m; one of the most common phyllidiid on Indo-Pacific reefs; the flatworm *Pseudoceros imitates* mimics this species. Size: 4 cm |
| 18-SM-N-22 | *Phyllidia coelestis* | Blue with black pigment between the ridges; tubercles and rhinophores yellow; a black line on the foot sole. Probably the most common and widespread species of *Phyllidia*; in the open on patch reefs or reef faces; egg mass flat. Size: 5 cm |
| 18-SM-S-11 | *Phyllidiopsis krempfi* | Pink with irregular, black, longitudinal lines that meander between the tubercles; long spicules visible through the translucent pink notum; medial tubercles compound with tips lighter than the body; rhinophores pink, black posteriorly. Crawling on reef flats in 10-15 m. Size: 4 cm |
| 18-SM-S-9 | *Reticulidia fungia* | Bluish-white with a few broad, smooth, orange ridges with white crests; black areas between the ridges; mantle margin with a grey-blue line. On reef slopes and walls in 20-40 m. Size: 3.5 cm |
| 18-SM-S-17 | *Phyllidiella cooraburran* | Pinkish with a network on dorsal black lines; very large, isolated dorsal tubercles with broad pink bases and elongate multi-compound apices; rhinophores black. On shallow reefs where it feeds on orange sponges. Size: 5 cm |

Note: * The identification based on Behrens and Coleman (2005)

**Table 2.** The abundance of nudibranch associated-bacteria

| Specimen | Number of associated bacteria | Prospective active isolate |
|---|---|---|
| *P. pipeki* | 12 | SMN-2-2 |
| *C. lochi* | 10 | SMN-3-1, SMN-3-7, SMN-3-77 |
| *C. lochi* | 10 | - |
| *C. lochi* | 12 | - |
| *P. fissurata* | 13 | - |
| *C. lochi* | 12 | SMN-10-2, SMN-10-23 |
| *C. annae* | 15 | SMN-14-13 |
| *P. pustulosa* | 8 | SMN-19-12 |
| *P. coelestis* | 8 | SMN-22-13 |
| *C. lochi* | 13 | - |
| *P. krempfi* | 12 | - |
| *Reticulidia fungia* | 9 | SMS-9-15 |
| *P. cooraburrama* | 11 | SMS-17-9 |
| Total | 145 | 11 isolates |

Note: - : there is no activity were observed

**Table 3.** Antibacterial activity of prospective isolates against pathogenic bacteria at concentration 500 μg/mL

| Isolate | Positive control | Negative control | MRSA | | EC | | BS | | KP | | ML | | ESBL-EC | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| SM-N-19-12 | + | - | - | - | + | - | - | - | - | - | - | - | - | - |
| SM-S-17-9 | + | - | - | - | + | - | - | - | - | - | - | - | - | - |
| SM-N-22-13 | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SM-N-10-23 | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SM-N-3-1 | + | - | - | - | + | - | - | - | - | - | - | - | - | - |
| SM-N-14-13 | + | - | - | - | - | - | - | + | - | - | - | - | - | - |
| SM-N-10-2 | + | - | - | - | - | + | - | - | - | - | - | - | - | - |
| SM-N-3-77 | + | - | - | - | - | + | - | - | - | - | - | - | - | - |
| SM-S-9-15 | + | - | + | - | + | - | + | - | + | - | + | - | + | - |
| SM-N-2-2 | + | - | - | - | - | - | - | - | - | - | - | - | - | - |
| SM-N-3-7 | + | - | - | - | - | + | - | + | - | + | - | - | - | - |

Note: *1: biomass methanol extract, 2: broth ethyl acetate extract. +: positive reaction, -: negative reaction). Positive control: Vancomycin, negative control: DMSO. Activity screen with following microorganisms; MRSA (Methicillin-Resistant *Staphylococcus aureus*); EC (*Escherichia coli*); BS (*Bacillus subtilis*); KP (*Klebsiella pnemoniae*); ML (*Micrococcus luteus*); ESBL-EC (Extended-spectrum beta-lactamases-*Escherichia coli*).

Examination of the diversity of nudibranch-associated bacteria has rarely been done before. As a predator of other invertebrates such as ascidians, cnidarians, and sponges, the abundance of nudibranch-associated bacteria is influenced by the diversity of the bacteria living in the prey. Schuett and Doepke (2013) found a type of bacterial aggregate in several species of nudibranchs that bear a resemblance to the typical coccoid endobacterial that exists in their prey. In addition, Zhukova and Eliseikina (2012) stated that the feeding behavior of nudibranchs on sponges and tunicate leads to direct microbial transferal from the prey to the nudibranch's tissue. It was suggested that the nudibranch-associated bacteria in this study might have originated from its prey. However, the original associated-microorganisms from this invertebrate are poorly understood. Therefore, further study is strongly suggested to confirm the bacteria's origin. As depicted in Table 2, 11 prospective isolates showed antibacterial activity. A previous study of Böhringer et al. (2017) also proved that nudibranch-associated bacteria in Indonesia are active in the production of antibacterial compounds.

**Antibacterial activity of the crude extract**

The active isolates were cultured for production of the extract that also be screened against all of the pathogens. The test exhibited that six isolates produced the extract that showed activity against one pathogen, and two isolates produced extract that was active against two pathogens (Table 3.). Methanol and ethyl acetate were used for extraction to provide the polar and less-polar compounds, respectively (Jassbi et al. 2016).

The extract of the isolates SM-S-9-15 and SM-N-3-7 showed broad-spectrum antibacterial activity due to its ability to inhibit more than two pathogenic bacteria. Meanwhile, the isolates that were active against one pathogen were SM-N-19-12, SM-S-17-9, SM-N-10-23, SM-N-14-13; SM-N-10-2, and SM-N-3-77 (see Table 3). The antibacterial property of nudibranch-associated bacterial crude extract has been investigated by Böhringer et al. (2017). They reported that among 49 isolated bacterial strains, 35 isolates (71.42%) showed antibiotic activity. They additionally support the notion that nudibranch-associated bacteria is a promising source for antibiotic discovery. Previously, Riyanti et al. (2009) gave a similar result in that a nudibranch-associated actinobacteria, *Streptomycetes* sp. from Panjang island, Jepara, exhibited antibacterial activity against several MDR bacteria. In this study, only seven (4.83%) bacterial crude extracts showed antibacterial activity against MDR bacteria. This number was lower than a previous study by Böhringer et al. (2017). However, Riyanti et al. (2009) specifically looked at the ability of nudibranch-associated bacteria to combat MDR bacteria as opposed to Böhringer et al. (2017) who targeted non-MDR bacteria. Finally, Sibero et al. (2019b) proved that both intra- and extracellular metabolites from marine microorganisms performed antibacterial activity against MDR bacteria.

**Diversity of potential bacteria associated nudibranch**

This research reports that host and the specific of bacteria species influenced the potential bacteria as antibacterial activity. There were among others 11 bacterial isolate species (Figure 3) from the genus *Bacillus*, one species from the genus *Pseudoalteromonas*, one species from the genus *Vibrio* and eight species from the genus *Virgibacillus*. Previous research shows that genus *Pseudoalteromonas* isolated from marine sponge (*Mycale armata*) produces red pigments and has antibacterial activity (Feher et al. 2008). Isnansetyo and Kamei (2003), also reported antibacterial activity, namely a new anti-

MRSA phenolic composition of *Pseudoalteromonas*, isolated from seawater. Another study of anti-MRSA looked at the potential of symbiotic bacteria from marine organisms such as Tunicata (Fedders et al. 2010), Porifera (Kamei and Isnansetyo 2003), and marine algae (Hentschel et al. 2001). *Vibrio* sp. is bacteria symbiosis with animal species of *Dysilea* sp. found in the Indian Ocean. This bacterium produces bioactive ingredients bis (dibromophenyl) ether, which is typically found in *Dysilea* sp. This also proves that bacteria in this symbiosis obtain secondary metabolisms (Sidharta 2000). Based on literature review, this extension discusses that the bacteria *Virgibacillus* sp. has potential as a protease producer (Sinsuwan et al. 2010) and antifungal in gray mold disease caused by *Botrytis cinerea* fungi in strawberry plants (Essghaier et al. 2009).

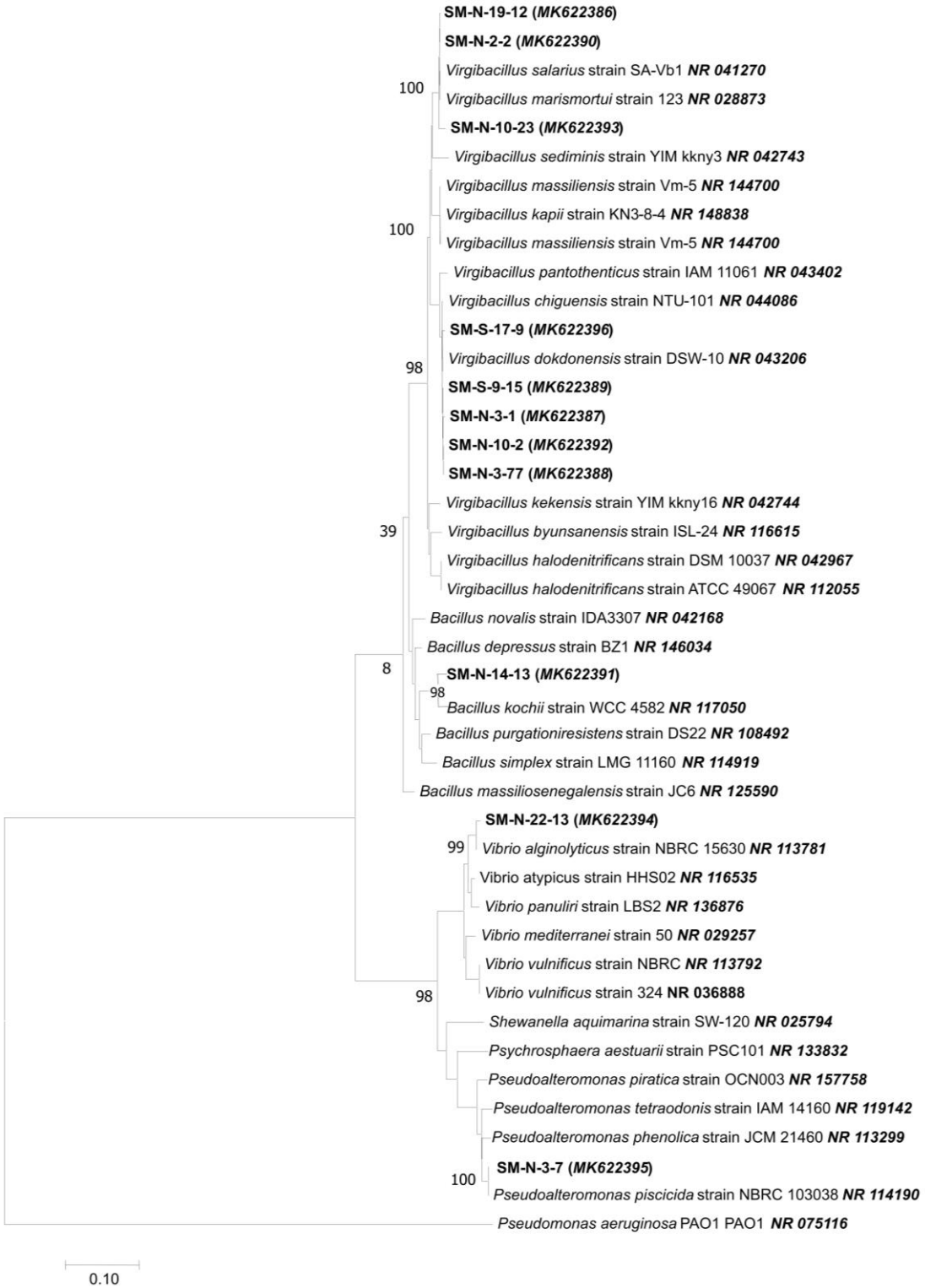


**Figure 3.** Phylogenetic tree of bacteria associated nudibranch

## ACKNOWLEDGEMENTS

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