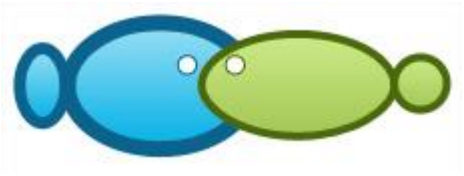

# Antibacterial activity of Gorgonian coral *Junceella* associated bacteria against catheter associated urinary tract infection (CAUTI) pathogens isolated from Karimunjawa Marine National Park, Java Sea, Indonesia

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**Abstract**. Catheter-associated urinary tract infection (CAUTI) is the most common nosocomial infection in hospitals worldwide. CAUTI treatment using antibiotics leads to the resistance of pathogenic bacteria against several classes of antibiotics. Hence, the search for new antibiotic compounds is carried out continuously. This research aimed to obtain the Gorgonian coral *Junceella* associated bacteria with antibacterial activity against CAUTI pathogens. The antipathogenic assay using the agar plug method showed that 2 of the 14 isolates, SA19.2 and SA19.3, had activity against *Escherichia coli* and *Klebsiella pneumoniae*. Detection of biosynthetic gene clusters indicated the presence of the NRPS gene in both active isolates. The 16S rRNA gene sequence analyses showed that SA.19.2 and SA.19.3 isolates were closely related to *Micrococcus yunnanensis*.
**Key Words**: antimicrobial activity, diversity, Karimunjawa islands, UTIs pathogens.

**Introduction**. Urinary tract infection (UTI) occurs in the urinary tract, from the urethra to the kidneys (Ramli 2020). UTI is one of the most common nosocomial infections found in various hospitals. Approximately 40% of all cases of nosocomial infection are UTIs, of which 80% are caused by catheter insertion (CAUTI) (Hariati et al 2019). In the case of CAUTI, indwelling catheter placement can increase the risk of infection by 3 to 10% daily due to bacterial colonization of the urine collection bag (Advani & Fakih 2019; Flores-Mireles et al 2019). The most commonly reported causative agents of CAUTI are *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*. Antibiotics, such as ciprofloxacin, levofloxacin, and ceftriaxone are used to treat CAUTI (Weiner et al 2016). However, long-term use of antibiotics can lead to the resistance of pathogenic bacteria against antibiotics (Aslam et al 2018). This indicates the need of new antibiotic compounds.

Karimunjawa Marine National Park is one of the marine protected areas in Indonesia. Karimunjawa has a high diversity of marine biological resources that have the potential as a source of new antibiotic compounds. One of these biological resources are Gorgonian corals (Sulardiono et al 2017; Teffu et al 2020; Sabdono et al 2022). Previous studies have reported that *J. juncea* extract has antibacterial activity against *E. coli*, *Salmonella typhii*, *Shigella flexneri*, and *Vibrio cholerae* (Kumar et al 2012). However, excessive use of Gorgonian corals in the production of antibiotics can cause environmental damage (El Samak et al 2018). To overcome this problem, research can be carried out on bacteria associated with Gorgonian corals. Previous studies have reported that crude extracts of bacteria associated with *J. juncea* are known to have antibacterial activity

against *E. coli*, *Bacillus subtilis*, and *Staphylococcus aureus* (Gao et al 2010). The antibacterial activity of these bacteria can be suspected due to the presence of a biosynthetic gene cluster (Martinet et al 2019). In addition, the antibacterial activity of bacteria associated with Gorgonian corals was published in a few studies. Therefore, this study aims to obtain bacteria associated with *Junceella* sp. from Karimunjawa, which has antibacterial activity against CAUTI pathogens through a polyphasic approach.

## Material and Method

***Sampling***. Gorgonian coral samples were collected from Sambangan Island (S 5°50'27.8"; E 110°34'54.8), Seruni Island (S 5°51'13.3"; E 110°34'36.8"), and Burung Island (S 05°53'27.9"; E 110°20'46.2"), Karimunjawa Marine National Park, Jepara, Central Java, Indonesia, in March 2021 (Figure 1). Environmental parameters including temperature, salinity, and pH were measured *in situ* using a GMK-910T thermometer, hand refractometer ATAGO MASTER 20 M, and a pH meter AZ 8682, respectively.

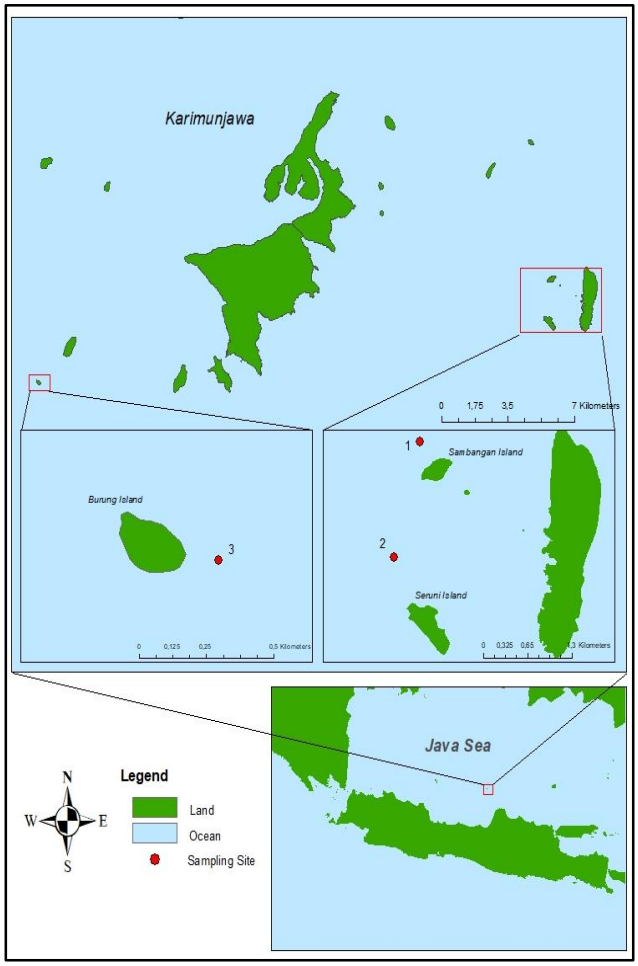


Figure 1. Sampling site location.

Sampling was carried out by scuba diving at a depth of 3-5 m. The samples were identified based on their morphology by referring to Tuti & van Ofwegen (2018). Analyses were conducted in the Tropical Marine Biotechnology Laboratory, Diponegoro University, Semarang, Indonesia.

***Bacterial isolation***. The serial dilution method was used to isolate bacterial symbionts. The samples were crushed with a mortar and pestle, then diluted with sterile seawater at three dilutions ($10^{-1}$, $10^{-2}$, $10^{-3}$). 1 mL of dilution $10^{-3}$ was inoculated on Zobell Marine Agar (ZA) by the spread plate method and incubated at 27°C for 24 hours. The bacterial isolates

to be purified were selected based on the different colony morphology. Purification was carried out on ZA media by streak method and incubated at 36°C for 24 hours (Kristiana et al 2019).

***Screening of antibacterial activity***. Bacterial isolates were screened for their antibacterial activity by the agar plug method (Sibero et al 2018). All bacterial isolates were refreshed on ZA media and incubated for 3x24 hours at 36°C. After that, each bacterial isolate was cut into a circle shape and placed on Mueller Hinton Agar (MHA) growing pathogen and incubated at 36°C. Observations of bacterial isolates were carried out 24 hours and 48 hours after testing. Bacterial isolates that have antibacterial activity will form an inhibition zone around their colonies (Sibero et al 2018).

***Morphological characterization***. Morphological characterization was carried out macroscopically and microscopically, determining size, color, and gram staining of colonies (Sousa et al 2015; Becerra et al 2016).

***Biochemical test***. The biochemical tests carried out in this study consisted of enzyme tests, sugar reduction tests, citrate tests, $H_2S$ tests, and motility tests. Enzyme test was carried out using nutrient agar (NA) added with certain substrates, such as carboxymethyl cellulose for cellulase enzyme test, starch for amylase enzyme test, and tween 80 for lipase enzyme test. The catalase enzyme test was carried out using hydrogen peroxide ($H_2O_2$) dripped directly on the bacterial isolates (Remijawa et al 2020). Sugar reduction was carried out using triple sugar iron agar (TSIA) for testing glucose, lactose, and sucrose, and using nutrient broth (NB) with phenol red as an indicator for testing mannitol, fructose, and trehalose (Talaiekhozani et al 2015; Sura & Hiremath 2019). The citrate test was carried out using Simmon Citrate Agar (SCA) (Talaiekhozani et al 2015). $H_2S$ and motility tests were carried out using sulfide indole motility (SIM) (Islam et al 2017).

***Salinity test***. The salinity test was carried out by inoculating bacterial isolates on agar media with 0 ppm and 35 ppm salinity using the streak method. Bacterial isolates that can only grow in media with a salinity of 35 ppm are categorized as obligate marine bacteria. Meanwhile, bacteria that can grow in media with 0 ppm and 35 ppm salinity are categorized as facultative marine bacteria (Wijaya et al 2022).

***Molecular identification***. In this study, bacterial isolates were identified with molecular identification combined with classical methods (Johnson 2014). Species identification of active bacterial isolates was carried out using molecular identification. DNA extraction was carried out according to the Promega Universal Wizard KIT protocol. The phylogenetic tree was constructed using the Phylogenetic Analysis Using Parsimony (PAUP*) software 4.0 version.

***Detection of PKS I, PKS II, and NRPS genes***. Gene detection was carried out using specific primers consisting of PKS I (KSα-F and KSα-R) at 700 bp – 800 bp, PKS II (IIPF6 and IIPR6) at 600 bp – 650 bp, and NRPS (A2gamF and A3gamR) at 200 bp – 300 bp. Amplification was carried out using a PCR mix with the same composition as the DNA amplification in the previous step (Sibero et al 2019; Wijaya et al 2022).

***Crude extract production***. The crude extract production was carried out according to Sibero et al (2018), with some modifications. Isolates with antibacterial activity were cultured on ZA media and incubated for 7x24 hours at 36°C. Extraction of metabolites was carried out by the maceration method. Ethyl acetate solvent was added to the production medium in a ratio of 1:2 (media: solvent) and agitated using a shaker at a speed of 200 rpm for 1 hour. A rotary evaporator at 40°C was used to separate and concentrate the ethyl acetate phase for obtaining a crude extract.

***Antibacterial assay***. The antibacterial activity test was conducted by the disc diffusion method. Observations of the crude extract test were carried out 24 hours and 48 hours

after testing. The crude extract with antibacterial activity will form an inhibition zone around the paper disc (Wijaya et al 2022).

**Results and Discussion**. Gorgonian coral samples were obtained from 3 different sampling points, namely Sambangan, Burung, and Seruni Islands. The three gorgonian samples were pinky red color, formed whip-like colonies that were unbranched, and had club shaped sclerites with monomorphic polyps (Figure 2).


Figure 2. Gorgonian *Junceella* sp. samples from different island site locations; A - Sambangan Island; B - Burung Island; C - Seruni Island.

Previous studies have reported the existence of *Junceella* sp. in several islands of Indonesia such as East Kalimantan, East Nusa Tenggara, Sulawesi, and West Papua (Syahrir et al 2018; Teffu et al 2020). Karimunjawa waters have a fairly high diversity and abundance, presumably because its waters are protected areas and have a good coral reef ecosystem as a habitat for *Junceella* sp (Sulardiono et al 2017; Sabdono et al 2022). The values of aquatic environmental parameters at the time of sampling were 29.75°C for water temperature and 34.33 ppm for salinity, and are suspected to be one of the factors causing the abundance of *Junceella* sp. at the sampling location. This is following a previous study reporting that the optimal water parameter conditions for Gorgonian coral growth were a temperature of 23-30°C and a salinity of 30-35 ppm (Abdullah et al 2019).

The 14 purified isolates were screened for their antibacterial activity by the agar plug method. The results showed that 2 of the 14 isolates, SA19.2 and SA19.3, inhibited the growth of *E. coli* and *K. pneumoniae*, respectively (Table 1).

Table 1

Antibacterial activity of Gorgonian coral associated bacteria

| *Location* | *Total isolate* | *Percentage of active isolates* | *Isolate code* | *E. coli* | | *P. aeruginosa* | | *K. pneumoniae* | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *1* | *2* | *1* | *2* | *1* | *2* |
| Sambangan Island | 5 | (2) 40% | SA19.1 | - | - | - | - | - | - |
| | | | SA19.2 | + | + | - | - | - | - |
| | | | SA19.3 | - | - | - | - | + | + |
| | | | SA19.4 | - | - | - | - | - | - |
| | | | SA19.5 | - | - | - | - | - | - |
| Burung Island | 5 | (0) 0% | BU20.1 | - | - | - | - | - | - |
| | | | BU20.2 | - | - | - | - | - | - |
| | | | BU20.3 | - | - | - | - | - | - |
| | | | BU20.4 | - | - | - | - | - | - |
| | | | BU20.5 | - | - | - | - | - | - |
| Seruni Island | 4 | (0) 0% | SE14.1 | - | - | - | - | - | - |
| | | | SE14.2 | - | - | - | - | - | - |
| | | | SE14.3 | - | - | - | - | - | - |
| | | | SE14.4 | - | - | - | - | - | - |

Note: +: growth inhibition; -: no inhibition.

These two isolates, SA19.2 and SA19.3, were characterized microbiologically by using serial methods adopted from Islam et al (2017) and Remijawa et al (2020). The morphological characterization of two active bacterial isolates showed similar characters, specifically in the size, shape, color, and gram (+) of bacterial colonies. Physiological tests showed that both isolates had a positive reaction to the catalase and lipase enzymes. However, only SA9.2 showed a positive sugar reduction of fructose, mannitol, lactose, and sucrose. The SA9.3 isolate showed positive sugar reduction of fructose only. Both isolates showed a negative reaction on citrate, $H_2S$, and to motility tests (Table 2).

Table 2

Biochemical characterization of bacterial active isolates

| *Biochemical test* | *Isolate code* | |
|---|---|---|
| | *SA.19.2* | *SA.19.3* |
| Enzyme | | |
| Catalase | + | + |
| Cellulase | - | - |
| Amylase | - | - |
| Lipase | + | + |
| Sugar reduction | | |
| Fructose | + | + |
| Mannitol | + | - |
| Trehalose | - | - |
| Glucose | - | - |
| Lactose | + | - |
| Sucrose | + | - |
| Gas production | - | - |
| Citrate | - | - |
| Motility | - | - |
| $H_2S$ | - | - |

Note: +: positive reaction; -: negative reaction.

The two active bacterial isolates could be classified as marine facultative bacteria due to growing in salinity concentrations of 0-35 ppm (Wijaya et al 2022). The presence of marine facultative bacteria in this study might be influenced by the rain cycle, drainage system, and human activities. These bacteria will adapt and survive in the marine environment (Tanod et al 2020).

Identification of bacterial species was carried out using a molecular method in the 16S rRNA region. The 16S rRNA sequence analysis showed that both SA.19.2 and SA.19.3 strains were closely related to *Micrococcus yunnanensis* (Figure 3). A previous study has reported that *M. yunnanensis* was isolated from deep-sea sediment samples (Hatmanti et al 2018). Interestingly, no studies have reported the presence of *M. yunnanensis* in association with Gorgonian corals. Detection of the biosynthetic gene cluster demonstrated the presence of the NRPS gene that was detected at 250 bp (Figure 4).

The presence of the NRPS gene in *M. yunnanensis* can be suspected as the cause of antibacterial activity in agar plug screening due to the production of antibacterial peptide compounds (Sibero et al 2019). However, the antibacterial activity test by the disc diffusion method on crude extracts showed that there was no inhibition zone formed by the crude extracts. This negative result in this study might be due to the solvent ethyl acetate (semi-polar), which cannot attract metabolites in the culture media (Hidayah et al 2016; Sibero et al 2018).

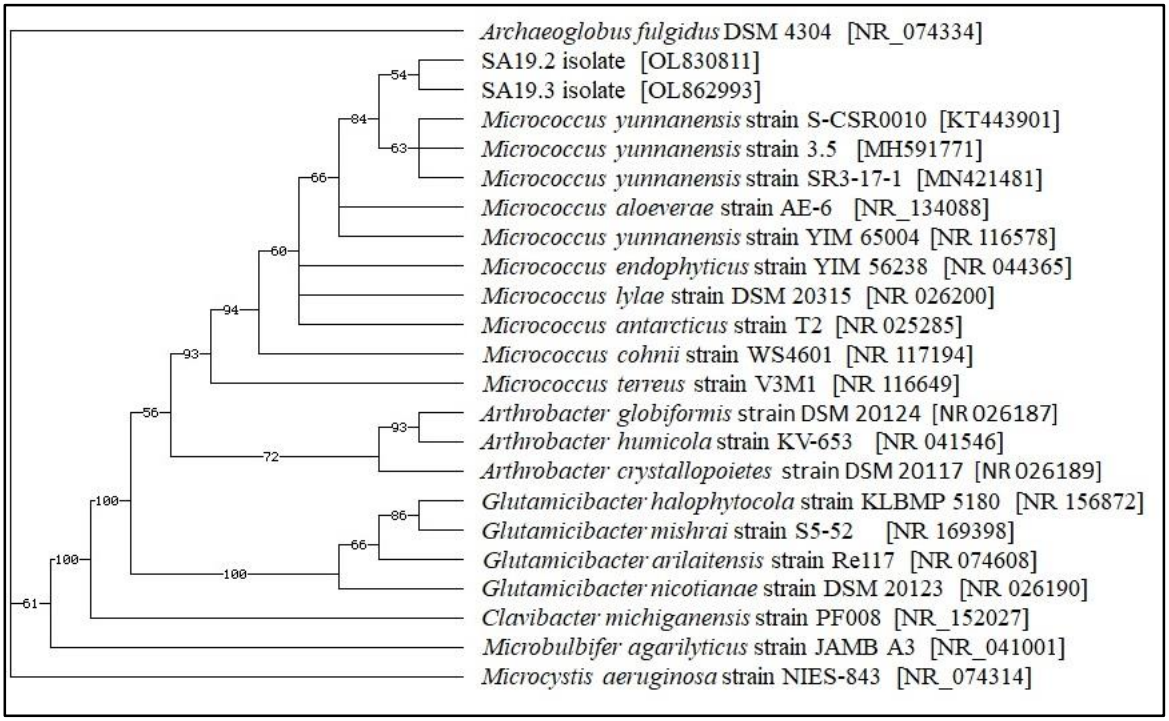


Figure 3. Phylogenetic tree of Gorgonian coral *Junceella* sp. associated bacteria SA19.2 and SA19.3 isolates with antibacterial activities. Bootstrap values (>50%) based on 1000 replicates are shown at branch nodes. *Archaeoglobus fulgidus* DSM 4304 was used as the outgroup.

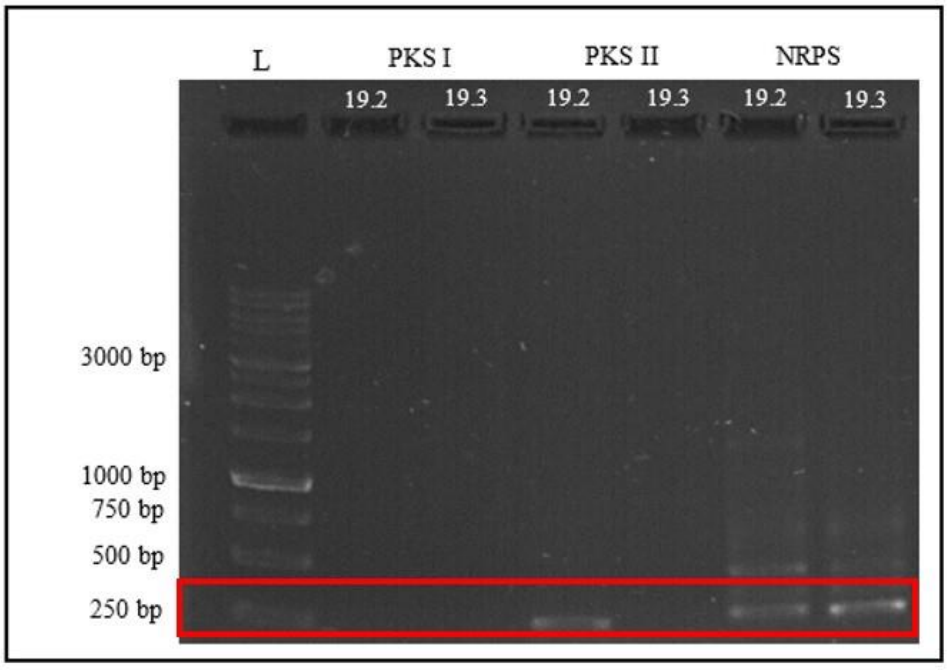


Figure 4. Detection of PKS I, PKS II, and NRPS genes on SA19.2 and SA19.3 isolates.

**Conclusions**. This study showed that the Gorgonian coral *Junceella* sp. associated bacteria, SA19.2 and SA19.3, have a broad spectrum of antibacterial activities. This result suggests that the Gorgonian *Junceella* sp. associated bacteria may represent a potential source of new antipathogenic compounds.

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**Conflict of Interest**. The authors declare that there is no conflict of interest.

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