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Antibacterial Activity of Gorgonian-Associated Bacteria from Jepara, Central Java, Indonesia

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A B S T R A C T

Gorgonian corals harbor diverse microbial communities, including bacteria with potential bioactive properties. This study aimed to isolate bacteria from gorgonian corals collected in Jepara waters, Indonesia, screen them for antibacterial activity against Klebsiella pneumoniae, and identify promising isolates with strong inhibitory effects. Marine Zobell Agar supplemented with nystatin was used for bacterial isolation. Tissue fragments of gorgonian coral were rinsed with sterile artificial seawater, excised, and inoculated onto the agar plates. Emerging bacterial colonies were subcultured to obtain pure cultures. The antibacterial activity of the isolates was evaluated against K. pneumoniae using the agar plug diffusion method. The isolate RAG 17.22 demonstrated consistent inhibitory activity against K. pneumoniae, as indicated by reproducible zones of inhibition. However, a reduction in inhibition zone diameter after 48 hours of incubation suggested a potential bacteriostatic mode of action. Further studies are required to characterize RAG 17.22 at the molecular level, including identification through 16S rRNA gene sequencing and chemical profiling of its secondary metabolites. The findings highlight the potential of coral-associated microbiota as a source of novel antimicrobial agents, particularly in the face of rising antibiotic resistance.

Keywords: antibacterial activity; *Staphylococcus aureus*; *Acinetobacter baumannii, gorgonian-associated bacteria*

1. Introduction

Gorgonian corals are important components of coral reef ecosystems that harbor diverse microbial communities, including bacteria with potential bioactive properties. The coastal waters of Jepara, Indonesia provide habitat for various gorgonian species, making it an ideal location to explore the antibacterial potential of gorgonian-associated bacteria. Investigating these bacteria is valuable for several reasons: 1. Gorgonians and their associated microbes may produce novel bioactive compounds with antibacterial activity against pathogens. 2. Studying gorgonian-bacteria interactions can provide insights into coral health and resilience. 3. Antibacterial compounds from marine sources could lead to new drug discoveries to combat antibiotic resistance. 4. Characterizing the antibacterial properties of gorgonian-associated bacteria contributes to our understanding of coral reef microbial ecology. This study aims to isolate bacteria from gorgonian corals collected in Jepara waters, screen them for antibacterial activity against common pathogens, and identify promising isolates with strong inhibitory effects. The findings will expand our knowledge of gorgonian microbial diversity in this region and highlight bacteria with potential biotechnological applications.

2. Material and methods

2.1 Preparation of Isolation Medium

Marine Zobell Agar was employed as the selective medium for bacterial isolation. To suppress fungal contamination, the medium was supplemented with the antifungal agent nystatin. All stages of bacterial isolation, purification, and maintenance were conducted on Marine Zobell Agar under aseptic conditions.

2.2 Isolation and Purification of Gorgonian Coral-Associated Bacteria

Tissue fragments of gorgonian coral were rinsed three times with sterile artificial seawater (ASW) to eliminate surface-adherent particulates and epibiotic contaminants, following the protocol of Sibero et al. (2019a). Subsequently, the tissues were aseptically excised using sterile surgical scalpels into ~1 cm segments. The sterilized tissue fragments were directly inoculated onto Marine Zobell Agar plates (Sabdono et al., 2019). To differentiate endogenous coral-associated bacteria from potential exogenous contaminants, uninoculated environmental control plates were included and incubated in parallel. All inoculated plates were sealed with parafilm or plastic wrap and incubated at ambient temperature (27 ± 1 °C). Emerging bacterial colonies were subcultured onto fresh Marine Zobell Agar plates through repeated streaking 2.to obtain pure cultures.

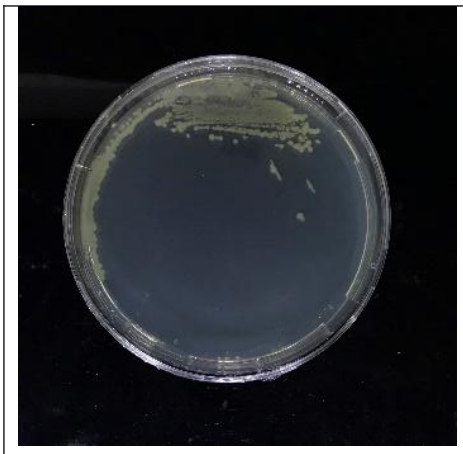
2.3 Antibacterial Screening Against Klebsiella pneumoniae

The antibacterial activity of coral-associated bacterial isolates was evaluated against *Klebsiella pneumoniae* using the agar plug diffusion method, as previously described by Sibero et al. (2017) and Ayuningrum et al. (2020). Initially, the isolates were pre-cultured on Marine Zobell Agar and incubated at room temperature (27 ± 1 °C) for 24 hours. Subsequently, the pure colonies were incubated on fresh Marine Zobell Agar plates for seven days to allow the accumulation of bioactive secondary metabolites.

After incubation, the bacterial biomass was transferred into Marine Zobell Broth and incubated for an additional 24 hours. The cultures were vortexed to ensure homogeneity, and the turbidity was adjusted to match a 0.5 McFarland standard, which corresponds to an approximate bacterial concentration of 1×10^7 to 1×10^8 CFU/mL (Quelab, 2005). The turbidity adjustment was conducted visually by comparing the suspension against a white background with printed black lines, following the procedure outlined by Aristyawan et al. (2018).

To prepare the assay plates, the surface of Mueller Hinton Agar (MHA) was swabbed evenly with the standardized suspension of K. pneumoniae using sterile cotton swabs. Agar plugs (~1 cm in diameter) containing the bacterial isolates were aseptically excised using sterile pipette tips and placed onto the inoculated MHA plates. The plates were sealed and incubated at 27 ± 1 °C for 24 hours. The presence of a clear inhibition zone surrounding the agar plugs was observed and recorded as an indication of antibacterial activity exhibited by the coral-associated bacterial isolates against K. pneumoniae (Ayuningrum et al., 2020).

3. Results



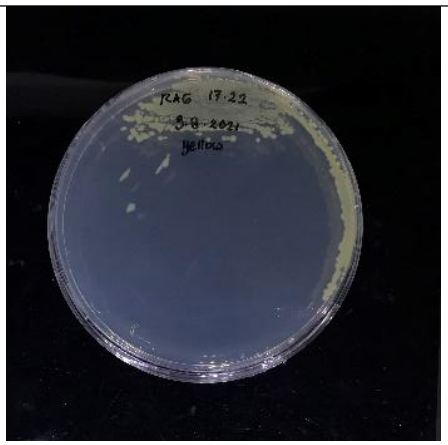
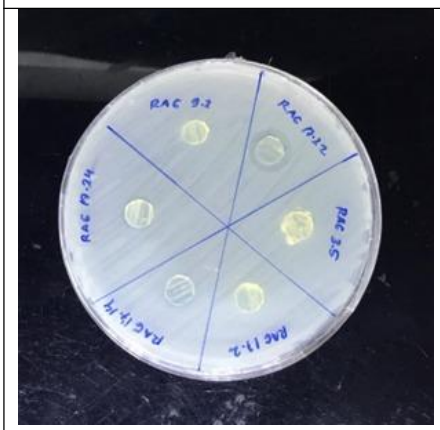


Figure 1. *Bacteria isolate RAG 17.22*



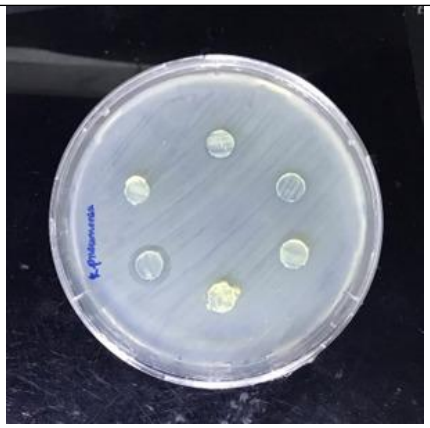


Figure 2.. *Antibacterial assay against Klesiella peumoniae*

4. Discussion

A primary mechanism by which gorgonian-associated bacteria contribute to coral defense is through the direct production of antimicrobial compounds that inhibit the growth of potential pathogens. The antibacterial screening of gorgonian coral-associated bacterial isolates was conducted to identify potential candidates capable of inhibiting the growth of *Klebsiella pneumoniae*. The clinical isolate of *K. pneumoniae* used in this study was obtained from infected patients at Kariadi General Hospital, Semarang, and represents a relevant target for bioprospecting of marine-derived antimicrobial agents.

The agar plug diffusion assay was employed to assess the antibacterial activity of the coral-associated isolates. This method is advantageous for preliminary screening as it allows the detection of extracellular antimicrobial metabolites diffused into the surrounding medium without the need for metabolite extraction (Sambanthamoorthy et al., 2011). The formation of a clear inhibition zone around the agar plug serves as an indicator of bioactivity and potential secondary metabolite production.

Among the tested isolates, RAG 17.22 demonstrated consistent inhibitory activity against *K. pneumoniae*, as indicated by reproducible zones of inhibition in both assay replicates. This observation suggests that the isolate actively secretes secondary metabolites with antibacterial properties into the surrounding agar matrix. Similar findings have been reported in previous studies on coral- and sponge-associated bacteria, which are known to be prolific producers of novel antimicrobial compounds (Wang *et al*., 2008; Radjasa et al., 2007).

However, a reduction in inhibition zone diameter was observed after 48 hours of incubation, indicating a potential bacteriostatic mode of action. Bacteriostatic agents inhibit bacterial growth without causing cell death, often through mechanisms such as interference with protein synthesis, membrane permeability, or metabolic pathway modulation (Pratiwi, 2017; Welsh, 2016). This is in contrast to bactericidal agents, which lead to bacterial cell death typically through disruption of cell wall biosynthesis or DNA damage. The transient nature of the inhibition observed in this study supports the hypothesis that the active compound(s) produced by RAG 17.22 function primarily through a bacteriostatic mechanism. Such mechanisms are clinically relevant, particularly in combination therapy or when host immune function is adequate to clear the infection once bacterial proliferation is halted (Kohanski *et al*., 2010)).

The formation of a clear zone is attributed to the presence of secondary metabolites in the extract, which are capable of damaging bacterial cell walls (Aristyawan et al., 2018). Secondary metabolites are compounds produced by organisms as a defense mechanism against competitive pressure, antagonistic interactions, infections, or predation by other marine organisms. These compounds are not essential for the organism's growth and development (Adamczak et al., 2020). The antibacterial mechanisms of secondary metabolites vary. Some secondary metabolites inhibit cell wall synthesis, thereby disrupting the formation of new bacterial cells. Others impair cell membrane function, leading to cell damage or death. Additionally, certain metabolites can interfere with protein synthesis, causing errors in mRNA translation and resulting in non-functional proteins. Inhibition of nucleic acid synthesis by these antibacterial compounds can also suppress bacterial growth.

The marine environment, especially coral reef ecosystems, has been recognized as a rich source of bioactive microbes with potential pharmaceutical applications. Marine-derived actinobacteria, pseudomonads, and other coral-associated taxa have been frequently reported to produce structurally diverse antimicrobial compounds, many of which are not found in terrestrial counterparts (Kellog *et al*., 2016). Further studies are required to characterize the isolate RAG 17.22 at the molecular level, including identification through 16S rRNA gene sequencing, as well as chemical profiling of its secondary metabolites using chromatographic and spectroscopic techniques. In addition, time-kill curve assays, minimum inhibitory concentration (MIC) testing, and cytotoxicity evaluation would provide deeper insight into its therapeutic potential and safety profile.

5. Conclusion

The isolate RAG 17.22 demonstrates promising antibacterial activity against *Klebsiella pneumoniae*, likely through a bacteriostatic mechanism. This finding underscores the potential of coral-associated microbiota as a reservoir of novel antimicrobial agents, particularly in the face of rising antibiotic resistance.

Conflict of the interest

The authors declare that there are no conflicts of interest.

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