Captive Corals as Sustainable Reservoirs of Bioactive Microbiome Bacteria: Collagen Scaffold Activation of Silent Biosynthetic Gene Clusters Enables Anti-MRSA Antibiotic Bioprospecting

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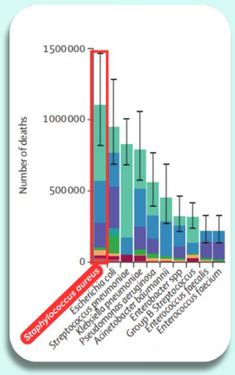
Background



(GBD Antimicrobial Resistance Collaborators, 2022)

Staphylococcus aureus is the leading cause of death related to antimicrobial resistence. In Indonesia an estimated of 40-50% *S. aureus* clinical isolate is thought to be MRSA (GBD Antimicrobial Resistance Collaborators, 2022).

The search for novel antibiotics increasingly focuses on marine symbionts, particularly coral-associated bacteria (Raina et al., 2016), whose genomes harbor diverse but mostly silent biosynthetic gene clusters (BGCs) for secondary metabolite production under conventional laboratory conditions (Timmermans *et al*., 2018).

One of the methods used to activate silent BGCs is subjection to a change in growth condition, such as adding chemical elicitors (Rutledge & Challis, 2015). Previous research found that adding scaffold structure can elicit silent BGCs in marine symbiont bacteria by acting as host-like physical and biochemical cues (Timmermans *et al*., 2018).

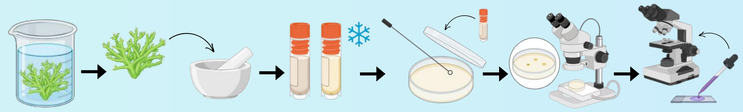
Objective

The objective of this research is to investigate whether a three-dimensional collagen scaffold could serve as an artificial host microenvironment to stimulate BGC expression and enhance anti-MRSA metabolite biosynthesis from Scleractinian coral symbionts. This study also investigate the effect of coral juice and cryopreservant type on Scleractinian-associated bacteria colony retrieval rate and growth.

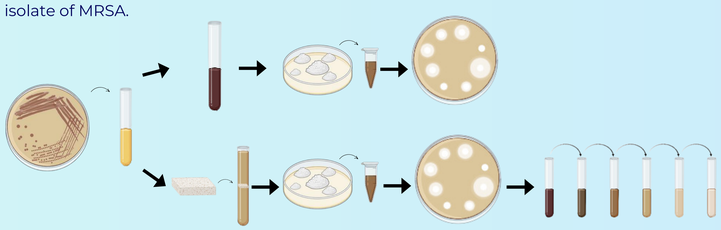
Methods

Five captive grown coral samples: *Acropora yongei*, *Caulastraea* sp., *Euphyllia* sp., *Fungia* sp., *Favites* sp., *Seriatopora* sp. originating from Indonesian aquarium trade.

Colony fragment was washed with sterile saltwater, homogenized, and cryopreserved (15% glycerol; 10% DMSO) and cultivated for bacteria on 1.2% Marine 2216 agar to be characterized morphologically. Colony retrieval rate of from different cryopreservant used was counted.



22 isolates were screened for methanol-soluble anti-MRSA metabolite. 5 isolates with anti-MRSA activity were grown with Marine 2216 broth enriched with collagen scaffold to be reextracted and retested through disk-diffusion assay followed with MIC assay on clinical isolate of MRSA.



The growth of 5 selected isolates were also characterized based on turbidity change over 7 days. The inhibitory and bactericidal capacity of methanol extract against MRSA was analyzed statistically using logistic regression and ANOVA.

Results & Discusssion

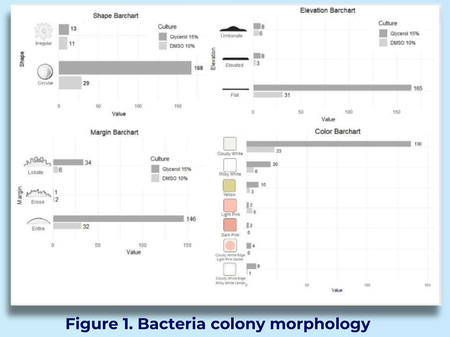


Figure 1. Bacteria colony morphology

Incubation on 1,2% Marine 2216 agar for 2 weeks at 28°C yielded many colonies characterized in Figure 1. No growth was observed on any control plates. Gram staining shows various bacteria cellular morphology, but molecular confirmation in future work is needed to identify the isolates. Only 5 distinct colonies from each sample were isolated and screened for anti-MRSA activity due to constraints.

Conclusion

This research demonstrates that aquacultured Scleractinian corals retain metabolically competent bacteria capable of producing potent anti-MRSA metabolites when stimulated by collagen scaffold. This integrative approach, which combines sustainable coral bioprospecting and ECM based elicitation provides a practical strategy for unlocking silent marine symbiont biosynthetic gene clusters while advancing antibiotic discovery and supporting the conservation of functional microbial biodiversity in ex situ systems.

Results & Discussion

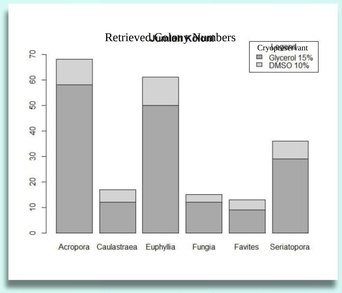


Figure 2. Colony retrieval rate between 15% glycerol and 10% DMSO stocks

15% glycerol acts as better cryopreservant for coral microbiome bacteria than 10% DMSO. Cryostock with 15% glycerol yields more cultivable colonies likely due to cytotoxic effect of DMSO, although the amount of cultivable bacteria varies greatly between coral species.

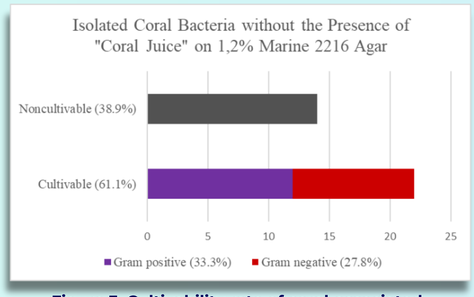


Figure 3. Cultivability rate of coral-associated bacteria isolates in relation to "coral juice"

About 38.9% of initial bacteria colonies can not be grown on conventional media (1,2% Marine 2216 agar). This indicates the need for "coral juice" found in the samples for metabolism of some coral microbiome bacteria. It is suspected that these bacteria are obligate symbiont to the coral microbiome.

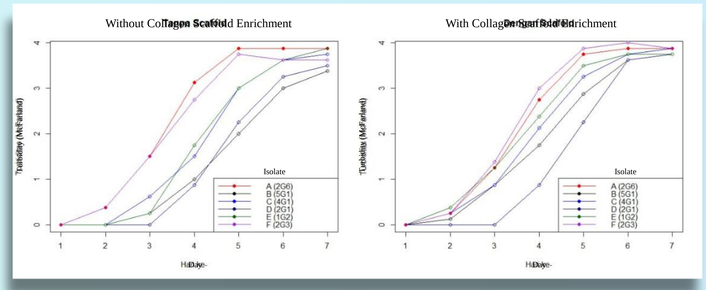


Figure 4. Growth curve of selected bacteria isolates in Marine 2216 broth without (left) and with (right) collagen scaffold enrichment

Collagen scaffold culture accelerated bacterial proliferation by 51.7%, reached higher stationary-phase density, and increased inhibitory activity by approximately fourfold (exp 1.3897, $p < 0.05$). One isolate exhibited a distinctive purple-black pigmentation exclusively in scaffold culture, suggesting the production of alternative metabolites and activation of previously silent biosynthetic pathways. The observed enhancement in antibacterial activity and phenotypic differentiation supports the hypothesis that scaffold-mediated spatial confinement and surface adhesion provide regulatory signals that activate silent BGCs through quorum sensing and stress-induced transcriptional derepression.

Acknowledgement

This research was funded by the ministry of higher education, science, and technology, government of Indonesia through Pekan Kreativitas Mahasiswa bidang Riset Eksakta (PKM-RE) research grant in 2023.

The authors also acknowledge support from Laboratorium Terpadu, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

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