Journal of Marine Research Vol 12, No. 2 Mei 2023, pp. 203-211 DOI : 10.14710/jmr.v12i2.35042
EISSN: 2407-7690
https://ejournal3.undip.ac.id/index.php/jmr

Antimicrobial Potential of Gorgonian *Alcyonium* sp. Associated Bacteria Against Human Pathogenic Skin Diseases

Avicenna Wijayanto[1], Agus Sabdono[2]*, Endang Sri Lestari[2], Mada Triandala Sibero[2]
[1]Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Diponegoro
Prof. Jacub Rais St., Semarang, Central Java, Indonesia
[2] Microbiology Department, Faculty of Medicine, Universitas Diponegoro
Prof. Soedarto S.H. St., Semarang, Central Java, Indonesia
*Corresponding author, e-mail : agus_sabdono@yahoo.com

ABSTRACT: Skin diseases are common in tropical countries due to microbial pathogens. The use of antibiotics is one of the efforts to treat skin diseases. However, the misuse and overuse of antibiotics can stimulate the emergence of multi-drug resistance (MDR) pathogens. This study focused on obtaining gorgonian-associated bacteria with antimicrobial activity against *Cutibacterium acnes*, *Staphylococcus epidermidis, Malassezia furfur,* and *Candida albicans*. This research procedure includes sampling, isolation, and purification of bacteria, antimicrobial test with agar plug method, morphological characterization, and molecular identification. The study results showed that one isolate of gorgonian-associated bacteria *Alcyonium* sp. from Seruni Island, Karimunjawa has antimicrobial activity against *Cutibacterium acnes, Staphylococcus epidermidis*, and *Candida albicans*. The results of molecular identification showed that isolate SE.4.2 had a similarity of 98.96% with *Pseudoalteromonas shioyasakiensis*.

Keywords: skin diseases, gorgonian-associated bacteria

INTRODUCTION

The human skin is the first body part that contacts the environment. The skin has an important role as the protector of the underlying body tissues. Human skin is the part of the body that covers the entire body and is the first tissue in contact with the environment, including with pathogenic microbes, making it susceptible to skin diseases. Skin diseases are common in tropical countries such as Indonesia and can be caused by microbial pathogens, temperature, lack of awareness, and bad sanitation. Skin diseases tend not to be fatal, so their presence is often ignored, but if left untreated, they will spread and be difficult to treat (Pardiansyah, 2015; Sahala *et al.,* 2016; Sundari *et al.,* 2018).

Skin microflora is microorganisms living on the skin of multicellular organisms such as humans with commensal symbiosis. However, if the balance between the skin and microflora is disturbed, the microflora can cause skin diseases (Byrd *et al.,* 2018; Resende and Carvalho, 2019). One skin disease often found is acne caused by infection with *Cutibacterium acnes* and *Staphylococcus epidermidis*. Acne is the eighth most common skin disease globally and affects about 10% of the world's population. Both *C. acnes* and *S. epidermidis* are microflora found in skin and mucus tissue (McLaughlin *et al.,* 2019; Nakase *et al.,* 2014). Other skin diseases can be caused by yeast infections, such as *Malassezia furfur* and *Candida albicans*. Yeast infection by *Malassezia furfur* is the general cause of dandruff, while *Candida albicans* cause a red rash (Kashem and Kaplan, 2016; Vest and Krauland, 2021). The use of antibiotics is one of the efforts to treat skin diseases. However, the problem is the emergence of pathogenic bacteria and fungi resistant to antibiotics, resulting in the emergence of multi-drug resistance (MDR) pathogens (Lim *et al.,* 2018; Magiorakos *et al.,* 2012). Therefore, an alternative antimicrobial that can overcome the multidrug resistance (MDR) pathogen is needed.

A total of 5.9% of marine natural compounds found in Indonesia have antibacterial activity. 15.8% of marine natural products that produce bioactive compounds are from phylum Cnidaria. Coral is a marine biota known for its potential for antimicrobial activities (Hanif *et al.,* 2019). Octocorals such

Diterima : 07-07-2022 ; Diterbitkan : 28-04-2023

Journal of Marine Research Vol 12, No. 2 Mei 2023, pp. 203-211

as sea pens, soft corals, and gorgonians live in symbiosis with other organisms such as Symbiodiniaceae, coralloid, fungi, Archaea, and bacteria. Corals defend themselves by excreting metabolites, and bacteria play a significant role in coral survival (McCauley *et al.,* 2020). This metabolite may contain an antimicrobial compound. However, determining the antimicrobial activity of gorgonian coral compounds will require large numbers of coral, so it can damage the coral reef ecosystem. The steps can be done to examine the activity of bacteria associated with gorgonian corals. Gorgonian *Alcyonium* is known to have potential metabolites that have active compounds (Abdel-Lateff *et al.*, 2019). Therefore, this study focused on obtaining antimicrobial compounds from gorgonian-associated bacteria in Karimunjawa waters.

MATERIAL AND METHODS

Gorgonian was sampled from Seruni Island waters by diving between 10-25 m depth (Figure 1). Oceanographic parameters include temperature, salinity, visibility, current, pH, and dissolved oxygen. The sample was documented and put inside a ziplock bag before storing it inside a cool box (Kristiana *et al.,* 2017; Wijaya *et al.,* 2021). Gorgonian was then identified according to Fabricius and Alderslade (2001). Isolation was carried out using the serial dilution method (10^{-1}, 10^{-2}) and inoculated on Zobell Agar (ZA) and Humic Acid Agar (HAA). The sample was then incubated at room temperature. Single colonies with different morphology were purified on Zobell Agar (ZA) and Actinomycetes Isolation Agar (AIA) using the streak method (Ayuningrum *et al.,* 2020; Kristiana *et al.,* 2020). Screening of antimicrobial activities was carried out using the agar plug method. Each was grown on Zobell Agar (ZA) and Actinomycetes Isolation Agar (AIA) using the full streak method and incubated for 3-7 days to reach maximum growth. Pathogenic microbes were cultured 24 hours before screening on Mueller Hinton Agar (MHA) for *Cutibacterium acnes* and *Staphylococcus epidermidis,* while *Candida albicans* and *Malassezia furfur* were cultured on Potato Dextrose Agar (PDA).

Antimicrobial activity was measured by observing a clear zone around the isolate (Balouiri *et al*., 2016; Sibero *et al*., 2019). Identification of bacterial morphology was carried out by macroscopic observation. Morphological characterization was carried out by observing the shape, margin, elevation, size, and color of bacterial colonies growing on agar media (Sousa *et al.,* 2015). Molecular identification was carried out following Zymo Quick DNA Miniprep Kit for DNA extraction. Bacterial identification was carried out using a 16S rRNA gene sequence. Amplification was performed using 27F and 1492R primers (Ayuningrum *et al.,* 2017). This primer is used because the 16S region is found in prokaryotes and has a long evolutionary process (Srinivasan *et al.,* 2015). The sequence was then identified based on NCBI BLAST. The isolate sequence and several sequences with close similarity were then analyzed for the phylogeny tree using MEGA X. Neighbor-joining method was used for phylogeny tree construction with bootstrap addition (Ayuningrum *et al.,* 2019).

RESULTS AND DISCUSSION

Gorgonian samples were obtained from Seruni Island, Karimunjawa, Java Sea. Environmental parameter such as pH, temperature, salinity, dissolved oxygen, current, and visibility was measured and shown in Table 1. The results show that the parameters are suitable for living coral optimally. The results of temperature measurements show the number 29.53°C, where the optimal temperature for corals to grow is a temperature of 23-30°C, h; however, corals can grow at the lowest temperature

Table 1. Environmental Parameters

Sampling Locations	Samples Code	Environmental Parameters					
		pH	Temperature (°C)	Salinity (‰)	DO (mg/L)	Current (m/s)	Visibility (m)
Seruni Island	SE.4	8,24	29,53	34,25	7,93	0,1	>4

Journal of Marine Research Vol 12, No. 2 Mei 2023, pp. 203-211

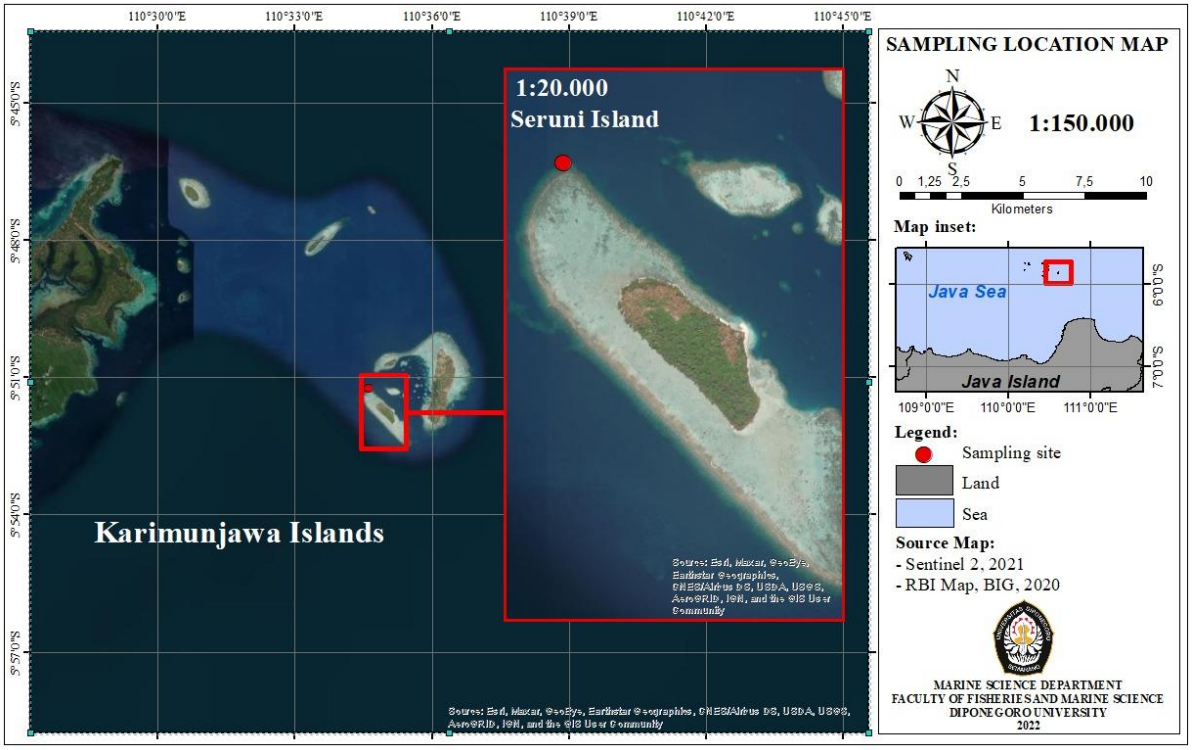

Figure 1. Sampling Location Site

Figure 2. *Alcyonium* sp. from Seruni Island

of 18°C and the highest temperature of 35°C. The measured salinity shows the25‰, where the ideal salinity for optimal living corals is at 30-35‰ (Dahuri, 2003). Gorgonian sampling results were identified based on their morphology. The gorgonian sample used was purplish-brown in h a thick branched cylindrical shape with a height of ±5.8 cm (Figure 2). Gorgonian species *Alcyonium* sp. was found in the intertidal zone under hard corals. Each polyp has eight tiny tentacles called pinnates, which contain stinging cells. *Alcyonium* sp. has thick branches resembling fingers (digitate)

Journal of Marine Research Vol 12, No. 2 Mei 2023, pp. 203-211

(Van Ofwegen *et al.,* 2007). Based on Fabricius and Alderslade (2001), the sample was presumed to be a gorgonian with the genus *Alcyonium* sp. This Gorgonian belongs to the phylum Cnidaria, Octocorallia, Alcyonacea and is widely distributed throughout the world's waters, and is an important component of coral reef ecosystems, especially in the Indo-Pacific region. There are 35 genera of these soft corals distributed in 15% of the area. Isolation of gorgonian-associated bacteria was carried out on two types of media, Zobell Agar (ZA) and Actinomycetes Isolation Agar (AIA) with the aim to obtain more diverse species of gorgonian-associated bacteria in each media. The number of isolates obtained on each ZA and AIA media was four colonies, with a total of eight colonies. It is suspected that the nutrients possessed by the two media are not optimal for growing association bacteria from gorgonians. Another type of media that can be used at the bacterial isolation stage is Humic Vitamin Agar (HVA), which is one of the enrichment media commonly used to isolate actinomycetes bacteria (Krishanti et al., 2018). In addition, the addition of CaCO3 to the media can be used to increase the growth of actinomycetes (Al-Ansari et al., 2020). Research conducted by Matsuyama et al. (2013) used a medium with 1-3% NaCl, a temperature of 28-30°C, and a pH of 6.5-8.0 to optimize the growth of bacteria isolated from marine sediments.The method used to test the antibacterial activity in this study is the agar plug method. This method is intended to obtain bacterial isolates that have antimicrobial compounds which are characterized by the presence of an inhibitory zone or clear zone around the agar plug (Balouri *et al*., 2016; Sibero *et al*., 2019). SE.4.2 was incubated for 3x24 hours with the aim that the isolate had achieved stationary growth. During its growth in the incubation phase, bacterial isolates were thought to produce secondary metabolites that diffused in the agar. Agar containing these secondary metabolites will then diffuse into the test medium, inhibiting the growth of pathogens and producing a clear zone. The antimicrobial assay was conducted against *Cutibacterium acnes, Staphylococcus epidermidis, Candida albicans,* and *Malassezia furfur.* The result of the antimicrobial assay using the agar plug method can be seen in Table 2.

The isolate SE.4.2 was found to be active against the test pathogenic bacteria which were gram-positive bacteria. This is presumably due to the presence of lipopolysaccharide which predominates in compiling the cell wall structure of gram-negative bacteria in isolate SE.4.2. These lipopolysaccharides are hydrophobic, making their cell walls impermeable to lipophilic solutes. Meanwhile, gram-positive bacteria do not have lipopolysaccharides, making their cell walls susceptible to metabolites (Singh *et al.,* 2016). The antimicrobial test did not get the results of antimicrobial activity against the pathogen *Malassezia furfur*. Generally, *M. furfur* can be treated using ketoconazole, which is an antifungal medication. However, recent studies have shown the emergence of *M. furfur* strains that have resistance to these antifungals (Park *et al*., 2020). This resistance is thought to be the cause of the isolates of gorgonian association bacteria *Alcyonium* sp. in this study did not have antimicrobial activity against *M. furfur*. Other isolates that showed no activity is suspected to have no microbial activity against skin pathogens, but the secondary metabolites produced can fight other disease pathogens.

Table 2. Antimicrobial Activity of Gorgonian Associated Bacteria

Sampling Locations	Total Isolate	Active Isolate	Medium	Code Isolate	*C. acnes*	*S. epidermidis*	*C. albicans*	*M. furfur*
Seruni Island	8	1(12,5%)	Zobell	SE.4.1	-	-	-	-
				SE.4.2	-	-	-	-
				SE.4.3	-	-	-	-
				SE.4.4	-	-	-	-
			AIA	SE.4.1	-	-	-	-
				SE.4.2	+	+	+	-
				SE.4.3	-	-	-	-
				SE.4.4	-	-	-	-

Description: (+) : presence of clear zone; (-) : absence of clear zone

Journal of Marine Research Vol 12, No. 2 Mei 2023, pp. 203-211

The morphology of the SE.4.2 isolate was known by macroscopic observation. Morphological characterization was carried out by observing the shape, margin, elevation, size, and color of bacterial colonies growing on agar media. Morphological observations were made on agar media because the shape of bacterial colonies could be observed more clearly than in liquid media. Observations were carried out when the bacteria were pure and reached the stationary phase to ensure that the bacterial colonies grew completely (Sousa *et al.,* 2015). The results of the colony morphology characterization were shown in Table 3.

According to Van Teeseling *et al*. (2017), the morphology of bacteria is very diverse. This form affects biological functions such as nutrient acquisition, motility, dispersion, stress resistance, and interactions with other organisms. The characteristics of bacteria generally do not change but can be influenced by environmental conditions. Azman *et al*. (2018) stated that there are microorganisms that produce pigments as secondary metabolites for protection against ultraviolet radiation, oxidants, extreme temperatures, and drying. Pigments can also act as antimicrobials against other bacteria.

The 16S rRNA gene is used for the identification of bacteria at the species level because it can differentiate between closely related bacterial species. This is because the 16S rRNA gene is found in prokaryotes with specific variables so it is used as a taxonomic classification (Jo *et al.,* 2017; Wang *et al.,* 2015). The results of the identification of isolates using the BLAST database showed that isolate SE.4.2 had a homology level with *Pseudoalteromonas shioyasakiensis* of 98.96% (Table 4). The similarity of the sequences of all isolates with the comparison sequence of more than 97% indicates that the isolates can be identified as the same species (Stackebrandt and Goebel, 1995).

Previous research by Matsuyama *et al.* (2013) stated that *P. shioyasakiensis* was isolated from marine sediments. The strain is a gram-negative, motile, rod-shaped, facultative aerobic or anaerobic bacterium, non-denitrifying, having ubiquinone-8 as the main respiratory quinone and requiring Na+ to grow.

Previous research by Matsuyama *et al.* (2013) stated that *P. shioyasakiensis* was isolated from marine sediments. The strain is a gram-negative, motile, rod-shaped, facultative aerobic or anaerobic bacterium, non-denitrifying, having ubiquinone-8 as the main respiratory quinone and requiring Na+ to grow. Bacteria from the genus *Pseudoalteromonas* are widely distributed in nature and can adapt to marine environments such as coastal waters, open and deep waters, and sediments (Ivanova *et al.*, 2014; Matsuyama *et al*., 2013). *Pseudoalteromonas* genera is often found associated with other organisms such as corals, sponges, molluscs, fish, tunicates, even in seawater

Table 3. Morphology Characterization

Morphological Characterization	Isolate Code
	SE.4.2
Color	Cream
Shapes	Circular
Margin	Entire
Elevation	Convex
Size	Small
Aerial hyphae color	-
Substrate hyphae color	-

Table 4. Molecular Identification of Active Isolate from Gorgonian Associated Bacteria

Isolate Code	*Closets Similarity*	*Percentage Identify*	*Query Cover*	*Accession*	*Accepted Accession*
SE.4.2	*Pseudoalteromonas shioyasakiensis*	98.96%	100%	NR_125458.1	OM108135

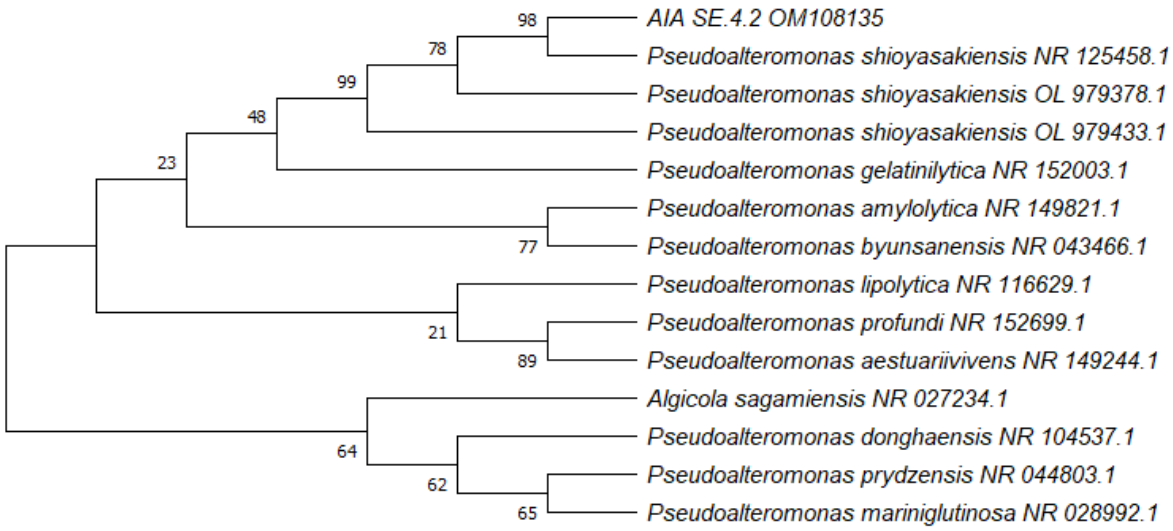

Figure 3. Phylogenetic-tree of Gorgonian-associated bacteria with antipathogen properties.

and sea ice. Several species of *Pseudoalteromonas* produce primary and secondary metabolites, including antibiotics, exopolymers, hydrolytic enzymes, and pigments. The study also found antifouling, antibiofilm, antibacterial, antifungal, algicide activities from the genus *Pseudoalteromonas* against pathogens (Atencio *et al.,* 2018; Supardy *et al.,* 2018; Wu *et al.,* 2017).The results of the phylogenetic tree construction at Figure 3 showed that the isolate SE.4.2 was still in the same group as *Pseudoalteromonas shioyasakiensis* because it had a similarity of 98.96%. Based on the results of the phylogenetic tree, the SE.4.2 sequence has a close relationship with *Pseudoalteromonas shioyasakiensis*, which means that in 1000 repetitions of the phylogenetic tree reconstruction, the SE.4.2 sequence has a 99% genetic relationship. Number 0.010 in Figure 2 means nucleotides per site in alignment, which shows the size of the genetic distance scale between species. So in 100 base pairs, there is 1 different nucleotide. *Algicola sagamiensis* was used in comparison as an outgroup species because it has a taxonomic genus level difference from the isolates used. *Algicola sagamiensis* is in separate roots from other isolates because it does not have similarities in the structure of the nucleotide base sequence with other sequences.

CONCLUSION

The SE.4.2 isolate has antimicrobial activity against *Staphylococcus epidermidis, Cutibacterium acnes,* and *Candida albicans.* Molecular identification showed that SE.4.2 isolate is closely related to *Pseudoalteromonas shioyasakiensis.*

ACKNOWLEDGEMENT

The author would like to thank to Karimunjawa National Park Agencies for the research permit. This research was funded by the Research and Public Service Institution, Diponegoro University through the WCR-UNDIP-B scheme, Contract no.118-04/UN76.1/PP/2021

REFERENCES

Abdel-Lateff, A., Alarif, W.M., Alburae, N.A., & Algandaby, M.M. 2019. Alcyonium octocorals: Potential source of diverse bioactive terpenoids. *Molecules*, 24(7):p.1370.

Al-Ansari, M., Kalaiyarasi, M., Almalki, M.A., & Vijayaraghavan, P. 2020. Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from

Journal of Marine Research Vol 12, No. 2 Mei 2023, pp. 203-211

Streptomyces sp. AS11 isolated from the marine environment. *Journal of King Saud University - Science*, 32(3):1993–1998. DOI: 10.1016/j.jksus.2020.02.005

Atencio, L.A., Dal Grande, F., Young, G.O., Gavilán, R., Guzmán, H.M., Schmitt, I., Mejía, L.C., & Gutiérrez, M. 2018. Antimicrobial-producing Pseudoalteromonas from the marine environment of Panama shows a high phylogenetic diversity and clonal structure. *Journal of basic microbiology*, 58(9):747-769.

Ayuningrum, D., Kristiana, R., Asagabaldan, M.A., Sabdono, A., Radjasa, O.K., Nuryadi, H., & Trianto, A. 2017. Isolation, characterisation and antagonistic activity of bacteria symbionts hardcoral Pavona sp. isolated from Panjang Island, Jepara against infectious multi-drug resistant (MDR) bacteria. *IOP Conference Series: Earth and Environmental Science,* 55(1): p. 012029. DOI: 10.1088/1755-1315/55/1/012029

Ayuningrum, D., Kristiana, R., & Asagabaldan, M.A. 2020. Potensi Bakteri Asosiasi Tunikata Sebagai Penghasil Senyawa Antibakteri Guna Menghambat Pertumbuhan Bakteri Multidrug Resistant. *Jurnal Pasir Laut*, 4(2):102-107.

Ayuningrum, D., Liu, Y., Sibero, M.T., Kristiana, R., Asagabaldan, M.A., Wuisan, Z.G., Trianto, A., Radjasa, O.K., Sabdono, A. & Schäberle, T.F. 2019. Tunicate-associated bacteria show a great potential for the discovery of antimicrobial compounds. *Plos one*, 14(3):e0213797.

Azman, A.S., Mawang, C.I. & Abubakar, S. 2018. Bacterial pigments: the bioactivities and as an alternative for therapeutic applications. *Natural Product Communications*, 13(12):p. 1934578X1801301240.

Balouiri, M., Sadiki, M., & Ibnsouda, S.K. 2016. Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6(2):71-79. DOI: 10.1016/j.jpha.2015.11.005

Byrd, A.L., Belkaid, Y., & Segre, J.A. 2018. The human skin microbiome. *Nature Reviews Microbiology*, 16(3):143-155.

Dahuri, R. 2003. Keanekaragaman Hayati Laut: Aset Pembangunan Berkelanjutan Indonesia. Gramedia Pustaka Utama, Jakarta.

Fabricius, K., & Alderslade, P. 2001. Soft corals and sea fans: a comprehensive guide to the tropical shallow water genera of the central-west Pacific, the Indian Ocean and the Red Sea. Australian Institute of Marine Science.

Hanif, N., Murni, A., Tanaka, C., & Tanaka, J. 2019. Marine natural products from Indonesian waters. *Marine drugs*, 17(6): p.364.

Ivanova, E.P., Jun, N.H., & Webb, H.K. 2014. The Family *Pseudoalteromonadaceae* In: Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F, editors. *The Prokaryotes*. 4nd edn, Gammaproteobacteria New York: Springer p. 575–582.

Jo, J.H., Kennedy, E.A., & Kong, H.H. 2016. Research techniques made simple: bacterial 16S ribosomal RNA gene sequencing in cutaneous research. *Journal of Investigative Dermatology*, 136(3):e23-e27.

Kashem, S.W., & Kaplan, D.H. 2016. Skin immunity to Candida albicans. Trends in immunology, 37(7): 440-450.

Krishanti, N.P.R.A., Zulfiana, D., Wikantyoso, B., Zulfitri, A., & Yusuf, S. 2018. Antimicrobial Production by an Actinomycetes Isolated from The Termite Nest. *Journal of Tropical Life Science*, *8*(3):279–288. DOI: 10.11594/jtls.08.03.10

Kristiana, R., Ayuningrum, D., Asagabaldan, M.A., Nuryadi, H., Sabdono, A., Radjasa, O.K., & Trianto, A. 2017. Isolation and partial characterization of bacteria activity associated with gorgonian Euplexaura sp. against methicillin-resistant Staphylococcus aureus (MRSA). *IOP Conference Series: Earth and Environmental Science*, 55(1): p.012056. DOI:10.1088/1755-1315/55/1/012056

Kristiana, R., Bedoux, G., Pals, G., Mudianta, I.W., Taupin, L., Marty, C., Asagabaldan, M.A., Ayuningrum, D., Trianto, A., Bourgougnon, N. & Radjasa, O.K. 2020. Bioactivity of compounds secreted by symbiont bacteria of Nudibranchs from Indonesia. *PeerJ*, 8:1-23. DOI: 10.7717/peerj8093

Lim, J.S., Park, H.S., Cho, S., & Yoon, H.S. 2018. Antibiotic susceptibility and treatment response in bacterial skin infection. *Annals of dermatology*, 30(2):186-191.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Lijequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., & Monnet, D.L. 2012. Multidrug-Resistant, Extensively Drug-Resistant And Pandrug-Resistant Bacteria: An International Expert Proposal For Interim Standard Definitions For Acquired Resistance. *Clinical microbiology and infection*, 18(3):268-281.

Matsuyama, H., Sawazaki, K., Minami, H., Kasahara, H., Horikawa, K., & Yumoto, I. 2014. Pseudoalteromonas shioyasakiensis sp. nov., a marine polysaccharide-producing bacterium. *International journal of systematic and evolutionary microbiology*, 64(Pt_1):101-106.

McCauley, M., Jackson, C.R., & Goulet, T.L. 2020. Microbiomes of Caribbean octocorals vary over time but are resistant to environmental change. *Frontiers in microbiology*, 11: p.1272.

McLaughlin, J., Watterson, S., Layton, A.M., Bjourson, A.J., Barnard, E., & McDowell, A. 2019. Propionibacterium acnes and acne vulgaris: New insights from the integration of population genetic, multi-omic, biochemical and host-microbe studies. *Microorganisms*, 7(5):p.128.

Nakase, K., Nakaminami, H., Takenaka, Y., Hayashi, N., Kawashima, M., & Noguchi, N. 2014. Relationship between the severity of acne vulgaris and antimicrobial resistance of bacteria isolated from acne lesions in a hospital in Japan. *Journal of medical microbiology*, 63(5): 721-728.

Pardiansyah, R. 2015. Association Between Personal Protective Equipment With Contact Dermatitis In Scavengers. *Jurnal Majority*, 4(4):80-87

Park, M., Cho, Y.J., Lee, Y.W., & Jung, W.H. 2020. Genomic multiplication and drug efflux influence ketoconazole resistance in Malassezia restricta. *Frontiers in cellular and infection microbiology,* 10: p. 191.

Resende, G., & de Carvalho, J.F. 2019. Human Microbiota and Autoimmune Diseases. Mosaic of Autoimmunity. pp. 191-201. Academic Press.

Sahala, M.A., Soedarman, S., Rizky, L.A., Natanegara, A P., Advani, M.S., & Sungkar, S. 2016. The prevalence of skin diseases and its association with hygiene behavior and level of education in a Pesantren, Jakarta Selatan 2013. *eJournal Kedokteran Indonesia*, p.119-24.

Sibero, M. T., Sabdaningsih, A., Radjasa, O. K., Sabdono, A., Trianto, A., & Subagiyo, S. 2019. Karakterisasi Senyawa Bioaktif Kapang Laut Trichoderma asperellum MT02 dengan Aktivitas Anti-Extended Spectrum β-Lactamase (ESBL) *E. coli. Jurnal Kelautan Tropis*, 22(1): 9-18.

Singh, V., Haque, S., Singh, H., Verma, J., Vibha, K., Singh, R., Vibha, K., Jawed, A., & Tripathi, C. K.M. 2016. Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. *Frontiers in microbiology*, 7:p.1921.

Sousa, A.M., Pereira, M.O., & Lourenço, A. 2015. MorphoCol: An ontology-based knowledgebase for the characterisation of clinically significant bacterial colony morphologies. *Journal of biomedical informatics*, 55:55-63.

Srinivasan, R., Karaoz, U., Volegova, M., MacKichan, J., Kato-Maeda, M., Miller, S., Nadarajan, R., Brodie, E. L., & Lynch, S.V. 2015. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. *PLoS ONE*, 10(2):1–22. DOI: 10.1371/journal.pone. 0117617

Stackebrandt, E. & Goebel, B.M. 1995. A Place for DNA-DNA Reassociation and 16S rRNA Sequence Analysis in The Present Species Definition in Bacteriology. *International Jurnal of Systematic Bacteriology,* (44):846– 849.

Sundari, J., Hamimah, P. Handayani, Yunita, Lesmono, I.D. Lesmono, M. Rahmayu, P. Kusumawardhani, Y. Budiarti, Fadilah & Nurhayati. 2018. Expert System Detection Human Skin Disease Using Forward Chaining Methode Based Web Mobile. MATEC Web Conf. Volume 218, 2018 The 1st International Conference on Industrial, Electrical and Electronics (ICIEE 2018) Article Number 02015 Number of page (s) 7 Section Control Electronics, Circuits, and Systems DOI : 10.1051/matecconf/201821802015 Published online 26 October 2018.

Supardy, N.A., Ibrahim, D., Nor, S.R.M., & Noordin, W.N.M. 2019. Bioactive compounds of Pseudoalteromonas sp. IBRL PD4. 8 inhibit growth of fouling bacteria and attenuate biofilms of Vibrio alginolyticus FB3. *Polish journal of microbiology*, 68(1):p.21.

Van Ofwegen, L.P., Häussermann, V., & Försterra, G. 2007. The genus Alcyonium (Octocorallia:

Journal of Marine Research Vol 12, No. 2 Mei 2023, pp. 203-211

Alcyonacea: Alcyoniidae) in Chile. *Zootaxa*, 1607(1):1-19.

Van Teeseling, M.C., de Pedro, M.A., & Cava, F. 2017. Determinants of bacterial morphology: from fundamentals to possibilities for antimicrobial targeting. *Frontiers in microbiology*, 8:p.1264.

Vest, B.E., & Krauland, K. 2021. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishig

Wang, X., Jordan, I.K., & Mayer, L.W. 2015. A phylogenetic perspective on molecular epidemiology. *Molecular medical microbiology*, 1:517-536.

Wijaya, A.P., Sibero, M.T., Zilda, D.S., Windiyana, A.N., Wijayanto, A., Frederick, E.H., Murwani, R., Wijayanti, D.P., Sabdono, A., Pringgenies, D. & Radjasa, O.K. 2021. Preliminary Screening of Carbohydrase-Producing Bacteria from Chaetomorpha sp. in Sepanjang Beach, Yogyakarta, Indonesia. *IOP Conference Series: Earth and Environmental Science*, 750(1): p. 012027. DOI: 10.1088/1755-1315/750/1/012027

Wu, Y.H., Cheng, H., Xu, L., Jin, X. B., Wang, C.S., & Xu, X.W. 2017. Physiological and genomic features of a novel violacein-producing bacterium isolated from surface seawater. *PLoS One*, 12(6): e0179997.