Study on the anti-vibrio activity of marine fungi *Aspergillus sydowii* and *Rhizopus* sp. using OSMAC Approach

Galank Fad'qul Janarkho[1], Agus Trianto[1*], Sri Sedjati[1], Rindiani Puja Listari[2]

[1]Department of Marine Sciences, Faculty of Fisheries and Marine Sciences,Universitas Diponegoro
Jl. Prof. Jacub Rais, Tembalang Semarang, 50275 Indonesia
[2]Institute of Marine Biology, National Taiwan Ocean University
No. 2, Beining Rd, Zhongzheng District, Keelung City, Taiwan 202, Republic of China
Email: agustrianto@lecturer.undip.ac.id

Abstract

Shrimp is one of the major aquaculture products in Indonesia. However, shrimp culture faces the peril of Vibriosis, a disease caused by the bacteria genus of *Vibrio*. Marine sponge-associate microbes are recognized for their potential as sources of antibacterial agent. The fungus *Aspergillus sydowii* isolate that used in this research originally isolated from marine sponge collected in Lampung Bay. The fungus was grown in various media e. g. Malt extract agar (MEA), MEA-Tempeh broth (MEA-T), and tofu dregs (TD) under various pH (5.5, 7.5, and 9.5). The fungus also be culture as mono-culture and co-culture with *Rhizopus* sp. The anti-vibrio assay was conducted using disk diffusion method. Based on anti-vibrio assay, the fungus *A. sydowii* and *Rhizopus* sp. didn't show any correlation with the anti-vibrio compound produced. The fungus *Rhizopus* sp. cultivated in tofu dregs media (TD) at pH 5.5 exhibited the highest potential for inhibiting against *V. alginolyticus* (5.85±0.24 mm), *V. harvey*i (5.20±0.20 mm), and *V. vulnificus* (4.33±0.15 mm), while the co-culture (*A. sydowii* and *Rhizopus* sp.) in TD media and pH 7.5 against *V. parahaemolyticus* (5.55±0.86 mm). The fungus cultured in pH 7.5 can promotes the potential inhibition zone than a pH 9.5.

Keywords: anti-vibrio, *A. sydowii*, *Rhizopus* sp., vibriosis, OSMAC.

INTRODUCTION

Aquaculture plays a significant role in food production. The potential of shrimp as the main product of Indonesian aquaculture contributes to the country's food resources. The Ministry of Marine Affairs and Fisheries stated that in the first quarter of 2022 shrimp aquaculture production reached 193,000 tons, an increase of 3% from 188,000 tons in 2021. However, disease outbreaks in marine aquaculture, such as vibriosis are a major concern for the Indonesian aquaculture sector. According to the Food and Agriculture Organization of the United Nations (FAO 2018), vibriosis infections cause global losses to exceed USD 3 billion annually.

Vibriosis is one of the threats to shrimp aquaculture. Vibriosis infection induces high mortality rates in shrimp cultivation (Shinn *et al*., 2018). *Vibrio* sp. inflicts attacks on shrimp at the larval and post-larval stages, resulting in mortality rates as high as 80% to 85% among the entire population (Aguirre-Guzmán *et al.*, 2013). In Asian shrimp aquaculture, for example, the financial impact of Acute Hepatopancreatic Necrosis Disease (AHPND) caused by vibrio infections in 2015 was enormous, reaching more than 26 million USD (Shinn *et al.*, 2018).

The excessive utilization of antibiotics in shrimp aquaculture resulting in bacterial resistance (Davies and Davies, 2010). The vibriosis pathogen has proven to be resistant to several commercial antibiotics, such as amplicilin, colistin, cephalothin, amoxycilin, carbencilin, ceftazidime, furazolidine, neomycin B, penicillin G, cephalexin, ciprofloxacin, nadilixic acid, and sulfamethoxazole (Elmahdi *et al.*, 2016)

Marine fungi are commonly live associated with other organisms such as sponges, algae, corals, and tunicates (Ramesh *et al.* 2021). Marine sponges are organisms with remarkable biological activity. Microorganisms that live in or around sponges are known to produce highly bioactive

*) Corresponding author
www.ejournal2.undip.ac.id/index.php/jkt
Diterima/Received : 04-08-2023, Disetujui/Accepted : 28-10-2023
DOI: https://doi.org/10.14710/jkt.v26i3.20451

substances such as fungi (Suryanarayanan, 2012). The marine fungi associated with sea sponges offer outstanding biological prospects, such as antiviral, antibacterial, and antifungal agents. According from previous research by Trianto *et al*., (2017a), the fungus *A. sydowii* has the capabilities as anti-fungal against *Malassezia furfur*, *Trichophyton* sp., and *Candida albicans*.

Rhizopus sp. known possesses antioxidant, antibacterial, and antifungal (El-Zawawy *et al*., 2023). The fungus from genus *Rhizopus* includes several species was used industrially for enzyme production (glucoamylases, cellulases, tannases), organic acids (lactic acid and fumaric acid), as well as traditional food production such as tempeh, peka, ragi, and loog-pang (Londono-Hernandez *et al*., 2017). Moreover, *Rhizopus* sp. exhibits ease of cultivation, rapid growth, and the ability to produce diverse secondary metabolites. Mambang *et al*., (2014), reported that the *Rhizopus* sp. from extract tempeh have activities to inhibit against *Bacillus subtilis* dan *Staphylococcus aureus.*

Several methodologies have been described to augment the range of bioactive compounds by activating dormant gene clusters, such as co-culturing, employing inducer molecules, and implementing the One Strain Many Compounds (OSMAC) approach (Romano *et al.*, 2018). The OSMAC concept is based on the assumption that changes in culture conditions might affect the biosynthetic profiles of microbial strains. This increases the diversity in the compound production of a single strain, which may lead to the discovery of new compounds or increased production of certain metabolites (Bode *et al.* 2002). The purpose of this research was to determine the anti-vibrio effectiveness of *A. sydowii* fungus by modulating its growth in various media and pH settings, followed by co-culturing it with *Rhizopus* sp.

MATERIALS AND METHODS

The fungus (L-11-02) was identified as *A. sydowii* was isolated from Lampung Bay water derived sponge (Trianto *et al.*, 2017b). *Rhizopus* sp. was isolated from fresh tempeh and purified onto MEA media. The anti-vibrio assay was conducted using *V. harveyi, V. alginolyticus*, *V. vulnificus*, and *V. parahaemolyticus,* strains obtained from the Marine Natural Product Laboratory, Diponegoro University.

Fungus was cultivated on 3 different media: Malt Extract Agar (MEA), MEA with extract tempeh's broth (MEA-T), and tofu dregs media (TD) at various different acidity (pH) range from 5.5, 7.5, and 9.5 incubated at temperature 28°C for 15 days. The tofu dregs media (TD) was prepared using 200 g of raw tofu dregs, 10 g of pure honey, 2 g of yeast powder, and 50 ml of seawater. The acidity (pH) was adjusted with a control solution containing 1M Potassium Hydroxide (KOH) and 1M Hydrochloric Acid (HCl) (technical grade). The fungal cultivation involved two different approaches: mono-culture and co-culture (*A. sydowi* and *Rhizopus* sp.).

The maceration method was used to extract the fungus (mono-culture and co-culture) after 15 days of incubation. Ethyl acetate was used as the solvent extraction (1:5 v/v) and solvent replacement was done for 3x24 hours (Sedjati *et al.*, 2020). The obtained filtrate was evaporated at 35°C using a Rotary Vacuum Evaporator (Eyela @N101, Tokyo, Japan).

The extracts were tested using the disc diffusion method (Kirby-Bauer) (Bahry *et al.*, 2021). Trypton Soy Agar (TSA) was utilized as the test media for the anti-vibrio assay. The bacterial density of the Nutrient Broth (HIMEDIA) was adjusted to a standardized value of 0.5 McFarland (equivalent to 1–2 x 10^8 CFU). Then, the tested bacteria swabbed onto the surface of Trypton Soy Agar (TSA) and allowed to sit for 3-5 minutes.

The extracts were injected into 6 mm Antimicrobial Susceptibility Disks (CT0998, OXOID) at the concentration of 1000 µg.disc^{-1}. Then, the disks were placed onto the TSA media and incubated for 24 hours. The presence of inhibition zones around the discs indicated a positive result. Observations were done after 24 hours, and the size of the inhibition zones measured using the Image J software.

Chloramphenicol was used as the positive control and Dimethyl sulfoxide (DMSO) as the negative control (Trianto *et al.*, 2017b).

RESULT AND DISCUSSION

The fungus *A. sydowii* (Figure 1) was confirmed based on its morphological characteristics that matches with previous molecular analysis (Trianto *et al*, 2017a). The fungus colony has a light greenish-brown colour. Under microscopic observation, the fungus has septate and branched hyphae, with conidia emerging from the swollen and thick-walled foot cells, bearing sterigmata. The conidia have a diameter of 2.5 µm with oval shape that attachs to phialides, which are connected to the ends of conidiophores. The conidiophores that exhibit a swelling and spreading is known as vesicle. The diameter of the conidia ranges from 2 µm to 4.4 µm (Soler-Hurtado *et al.,* 2016).

The fungus was grown in various media e. g. MEA, MEA-T, and TD under various pH (5.5, 7.5, and 9.5) for 15 days and extracted with maceration methods. The maceration use ethyl acetate as the solvent. Ethyl acetate has the ability to bind various compounds, including alkaloids, aglycones, flavonoids, glycosides, saponins, and steroids (Sari *et al*, 2021). Ethyl ecetate is known has the capability to attract a polar and non-polar compound (Makky *et al.*, 2021). The production of crude extract yields by mono-culture and co-culture (*A. sydowi* and *Rhizopus* sp.) shown in Figure 2.

The fungus *A. sydowii* cultured at TD media at pH 5.5. produced the highest amount of extract (606 mg/L medium), while the co-culture of *A. sydowii* and *Rhizopus* sp. gave the highest extract at TD media with pH 7.5 (942 mg/L medium). The peak production of the secondary metabolites occurs during the stationary phase of fungal growth on the day 15 that in a good agreement. The nutrient composition of the culture media influences the growth, multiplication, and synthesis of various secondary metabolites. Tofu dregs contains nutrients such as protein (16-24%), carbohydrates (52-56%), macro and trace mineral elements including Fe (200-500 ppm), Mn (30-100 ppm), Cu (5-15 ppm), Co (<1 ppm), and Zn (>50 ppm) (Kusumaningtyas *et al.*, 2020).

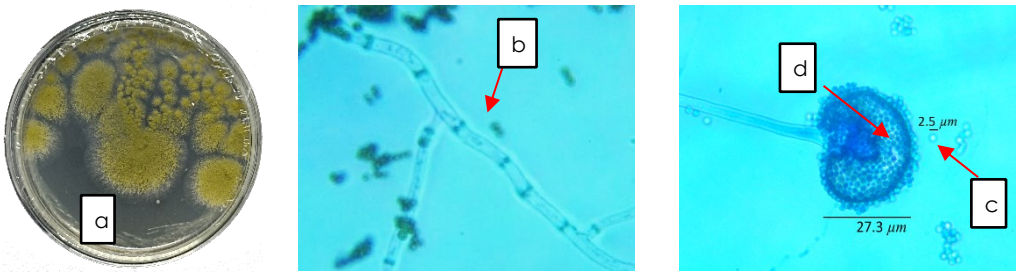

Figure 1. Morphology of *A. sydowii* at macroscopic and microscopic (AMScope M500, 400x) (a) the fungus colony on MEA medium (b) hifa (c) conidia (d) vesicle.

Table 1. Yield of crude extracts at various media and pH.

Strain	Crude extract yield (mg)								
	MEA[a]			MEA-T[a]			TD[a]		
	5.5[b]	7.5[b]	9.5[b]	5.5[b]	7.5[b]	9.5[b]	5.5[b]	7.5[b]	9.5[b]
A. sydowii	205.3	81.7	124	71.6	68.9	233.5	606.5	523.3	414.5
Rhizopus sp.	54.6	39.9	171.1	85.3	50.9	85.4	294.8	226.8	194
Co-culture	69.4	68.9	233.5	168.3	83.7	52	803.4	942.6	563

Note: a (media), b (pH); MEA= MEA seawater, MEA-T= MEA tempeh broth, TD= tofu dregs media.

The carbon sources can be obtained from carbohydrates, proteins, and lipids, while the nitrogen only derived from proteins. The secondary metabolites are achieved when the nitrogen is abundant and the carbon is low, highlighting the significance of the C/N ratio (Sedjati *et al.*, 2020). The essential roles of fungal metabolisms depend on the mineral and vitamin content when provide energy and act as a signaling molecule, facilitating enzymatic activity, supporting hemeproteins, contributing to cellular pigmentation, and participating in redox reactions (Walker and White, 2005).

According to the result, variations of pH resulted observable differences in the extent of the inhibition zone. The pH 7.5 exhibit a stronger inhibition zone compared than pH 9.5. The pH directly influences the rate of enzymatic activity in catalyzing reactions. pH can also affect enzyme activity and ion balance within biological systems. The influence of pH is attributed to the variations in charge of the amino acids comprising proteins. The biochemical processes in living organisms because most of the bioreactions involve the transfer of electrons due to the role of pH. The pH affects the permeability of cellular membranes and the transport of ions and molecules. The ionization state of molecules and the pH gradient across membranes are essential factors in regulating transport processes (Aoi and Marunaka, 2014). The ionization state and conformation of the active site of enzymes, influencing their catalytic efficiency (Schober *et al*., 2023). Hassan and Bakhiet (2017), reported that the fungus *A. fumigatus* cultured at pH 7, produces the strongest antibacterial compound tested against *Salmonella typhimurium, Listeria monocytogenes,* and *Pseudomonas aeruginosa*.

Based on the latest report, it is known that the fungus from genus *Aspergillus* has a capabilities and potential as anti-vibrio against *V. parahaemolyticus, V. harveyi, V. vulnificus, and V. alginolyticus* (Trianto *et al*., 2023). The compound synthesis by *Aspergillus* sp. known as *Aspergixanthones I* showed the strongest anti-vibrio activity against *V. parahemolyticus*, *V. anguillarum*, and *V. alginolyticus* (Zhu *et al.*, 2018). Another compound isolated from *A. sydowii is* produce a wide range of bioactive natural products e.g. polyketides, terpenoids, alkaloids, phenolic compounds, and peptides (He *et al*., 2018). Salvatore *et al*., (2018) reported that this fungus also produces mycotoxins, such as aflatoxins, ochratoxin A, patulin, and sterigmatocystin.

Surprisingly, based on the result from anti-vibrio assay, the *A. sydowii* didn't give the strong inhibition tested against vibrio bacteria. The fungus *Rhizopus* sp. cultivated in tofu dregs media (TD) at pH 5.5 exhibited the highest potential for inhibiting the growth of three bacteria: *V. alginolyticus* (5.85±0.24 mm), *V. harvey*i (5.20±0.20 mm), and *V. vulnificus* (4.33±0.15 mm), while the co-culture (*A. sydowii* and *Rhizopus* sp.) in TD media and pH 7.5 against *V. parahaemolyticus* (5.55±0.86 mm) (Table 2). This indicate that the inhibition zone formed is in the intermediate inhibition zone response (5 mm-10 mm) (Hasanuddin and Salnus, 2020). The use of tofu dregs media (TD) yielded the most prominent zones of inhibition. Tofu dregs, which is a by-product of tofu manufacturing exhibit a rich composition of fiber, protein, isoflavones (such as genistein and daidzein), lignans, phytosterols, saponins, and phytates (Sharma *et al.*, 2017). These components were utilized as a nitrogen source for the production of enzymes and lipids because the regulation of metabolite synthesis is primarily influenced by the C/N ratio (Sedjati *et al.*, 2020). Thus, makes the fungus cultivated on TD media exhibited the strongest anti-vibrio activities. The concentration of secondary metabolites in the TD extract was likely higher than in the other tested extracts, implying its potential to inhibit vibrio bacterial growth.

The co-culture yields lower results compared to mono-culture. When *Rhizopus* sp. is cultured individually, it demonstrates the capacity to generate bioactive compounds that possess inhibitory properties against four strains of vibrio bacteria. This implies that the co-cultivation of *A. sydowii* and *Rhizopus* sp. do not synergistically enhance the synthesis of secondary metabolites. Two or more organisms living together in the same ecosystem is a common phenomenon in nature. These corelation maybe be mutualism, antagonisms or just simply living together. Microorganisms were cultured together can contribute unique metabolic capabilities to each other (Carter and Shieh, 2015). The organism may produce certain precursor compounds required for the synthesis of bioactive

Table 2. Inhibition zones of ethyl acetate extracts against vibriosis bacteria

Strain	Media	pH	Inhibition zone (mm)			
			V. alginolyticus	*V. harveyi*	*V. parahaemolyticus*	*V. vulnificus*
A.sydowii	MEA	5.5	0.00±0.00	0.00±0.00	0.24±0.14	0.00±0.00
		7.5	0.44±0.03	0.00±0.00	0.29±0.10	0.00±0.00
		9.5	0.41±0.08	0.00±0.00	0.15±0.13	0.00±0.00
	MEA-T	5.5	0.49±0.16	0.00±0.00	0.00±0.00	0.00±0.00
		7.5	0.66±0.09	0.00±0.00	0.00±0.00	0.00±0.00
		9.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	TD	5.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		7.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		9.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Rhizopus sp.	MEA	5.5	0.45±0.18	0.00±0.00	0.19±0.19	2.04±0.31
		7.5	0.83±0.40	0.00±0.00	0.37±0.28	1.37±0.06
		9.5	1.04±0.25	0.00±0.00	0.57±0.05	0.00±0.00
	MEA-T	5.5	3.56±1.08	1.52±0.23	2.46±0.14	2.75±0.28
		7.5	1.92±0.51	0.93±0.10	1.77±0.20	3.09±0.80
		9.5	1.48±0.31	1.47±0.31	1.50±0.05	2.69±0.46
	TD	5.5	5.85±0.24	5.20±0.20	4.43±0.18	4.33±0.15
		7.5	5±0.52	5.35±0.25	4.40±0.35	4.11±0.68
		9.5	1.99±0.54	2.30±0.46	0.57±0.12	1.18±0.11
Co-culture	MEA	5.5	1.37±0.12	0.00±0.00	0.00±0.00	0.00±0.00
		7.5	1.61±0.12	0.00±0.00	0.00±0.00	0.00±0.00
		9.5	1.93±0.06	0.00±0.00	0.00±0.00	0.00±0.00
	MEAT	5.5	0.00±0.00	1.31±0.61	0.00±0.00	0.00±0.00
		7.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		9.5	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	TD	5.5	0.00±0.00	0.00±0.00	1.37±0.16	0.00±0.00
		7.5	3.45±0.61	2.40±0.29	5.55±0.86	1.8±0.12
		9.5	0.00±0.00	0.00±0.00	1.77±0.36	0.00±0.00

Note : ± (standart deviation); MEA= MEA seawater, MEA-T= MEA tempeh broth, TD= tofu dregs media.

molecules, while another organism may possess the enzymatic machinery required for the final conversion of these precursors to the desired bioactive compounds (Selegato *et al*., 2023). Sun *et al.* (2021), reported that the co-culture between *A. sydowii* and *B. subtilis* has developed a total of 25 newly biosynthesized metabolites. According to the study by Kobayashi *et al.* (1992), *Rhizopus* sp. produces protease enzymes during fermentation, which can hydrolyze proteins into peptides and impede the growth of *A. flavus*. Based on these results, it can be concluded that the co-cultivation of *A. sydowii* and *Rhizopus* sp. didn't effective to produce the secondary metabolite compounds that can inhibit the growth of vibriosis bacteria.

CONCLUSION

Surprisingly, the fungus *A. sydowii* didn't give the best inhibition against vibrio bacteria. The fungus *Rhizopus* sp. had the strongest anti-vibrio activity against *V. alginolyticus* (5.85±0.24 mm), *V. harveyi* (5.20±0.20 mm), and *V. vulnificus* (4.33±0.15 mm), while the co-culture had the strongest against *V. parahaemolyticus* (5.55±0.86 mm). The pH 7.5 exhibits a stronger inhibition compared than pH 9.5. Then, the co-culture didn't exhibit the best inhibition than a mono-culture.

ACKNOWLEDGMENT

This study is part of the final project of the master's program at Diponegoro University, Departement of Marine Science. The authors thanks Vidya Octaverina and Nining Nursalim for helping this research.

REFERENCES

Aguirre-Guzmán, G., López-Acevedo, E.A., & Vázquez-Sauceda, M.L. (2013). Efecto de *Vibrio harveyi* En La Sobrevivencia de Larvas de *Litopenaeus vannamei*. *Scientia Agropecuaria,* 4,121–27.

Aoi, W., & Marunaka, Y. (2014). Importance of pH Homeostasis in Metabolic Health and Diseases: Crucial Role of Membrane Proton Transport. *BioMed Research International*. 8 pages. doi: 10.1155/2014/598986.

Bode, H.B, Bethe, B., Höfs, R., & Zeeck, A. (2002). Big Effects from Small Changes: Possible Ways to Explore Nature's Chemical Diversity. *Chembiochem: A European Journal of Chemical Biology* 3(7), 619–27. doi: 10.1002/1439-7633(20020703)3:7<619::AID-CBIC619>3.0.CO;2-9.

Elmahdi, S., DaSilva, L.V., & Parveen, S., (2016). Antibiotic resistance of Vibrio parahaemolyticus and Vibrio vulnificus in various countries: a review. *Food Microbiology*, *57*, 128–134. doi: 10.1016/j.fm.2016.02.008.

Hasanuddin, A.R.P. & Salnus, S. (2020). Antibacterial Activity Of Clove Oil (*Syzygium Aromaticum*) in Inhibiting the Growth of *Streptococcus mutans* causing Dental Disease. *Bioma: Jurnal Biologi Makassar,* 5(2), 241-250

Hassan, S.A.A., & Bakhiet, S.E.A. (2017). Optimization of Antibacterial Compounds Production by *Aspergillus fumigatus* Isolated from Sudanese Indigenous Soil. *International Biological and Biomedical Journal,* 3(4), 3–8.

He, Y., Wang, B., Chen, W., Cox, R.J., He, J., & Chen, F. (2018). Recent advances in reconstructing microbial secondary metabolites biosynthesis in *Aspergillus* spp. *Biotechnology Advances,* 36, 739–783. doi: 10.1016/j.biotechadv.2018.02.001.

Kusumaningtyas, R.D., Hartanto, D., Prasetiawan, H., Triwibowo, B., Maksiola, M., Kusuma, A.D.H., Mezaki, N. M., Fidyawati, F., Mutaqin, A.M., & Loveyanto, R.O. (2020). The Processing of Industrial Tofu dregs Waste into Animal Feed in Sumurrejo Village, Semarang. *Rekayasa Jurnal Penerapan Teknologi Dan Pembelajaran,* 18(2), 36–41. doi: 10.15294/rekayasa.v18i2.27278.

Londono-Hernandez, L., Ramirez-Toro, C., Ruiz, H.A., Ascacio-Valdes, J.A., Aguilar- Gonzalez, M.A., Rodriguez-Herrera, R., & Aguilar, C.N. (2017). *Rhizopus oryzae* - Ancient microbial resource with importance in modern food industry. *International Journal of Food Microbiology*, 257, 110–127. doi: 10.1016/j.ijfoodmicro.2017.06.012

Makky, E.A., AlMatar, M., Mahmood, M.H., Ting, O.W., & Qi, W.Z. (2021). Evaluation of the Antioxidant and Antimicrobial Activities of Ethyl Acetate Extract of *Saccharomyces cerevisiae*. *Food Technology and Biotechnology, 59*(2), 127–36. doi: 10.17113/ftb.59.02.21.6658.

Mambang, D.E,P., Rosidah., & Suryanto, D. 2014. Aktivitas antibakteri ekstrak tempe terhadap bakteri *Bacillus subtilis* dan *Staphylococcus aureus*. *Jurnal Teknologi dan Industri Pangan,* 25(1), 115-118. doi: 10.6066/jtip.2014.25.1.115.

Ministry of Marine Affairs and Fisheries. (2021). Rilis Data Kelautan dan Perikanan Triwulan I Tahun 2022. Available at: https://kkp.go.id/sosek/artikel/40866-buku-rilis-data-kelautan-dan-perikanan-triwulan-i-2022. Accessced 25 July 2022.

Ramesh, C., Tulasi, B.R., Raju, M., Thakur, N., & Dufossé, L. (2021). Marine Natural Products from Tunicates and Their Associated Microbes. *Marine Drugs,* 19(6), 1–21. doi: 10.3390/md19060308.

Romano, S., Jackson, S.A., Patry, S., & Dobson, A.D.W. (2018). Extending the 'One Strain Many Compounds' (OSMAC) Principle to Marine Microorganisms. *Marine Drugs,* 16(7), 1–29. doi: 10.3390/md16070244.

Salvatore, M.M., Nicoletti, R., Salvatore, F., Naviglio, D., Andolfi, A. (2018). GC–MS approaches for the screening of metabolites produced by marine derived *Aspergillus*. *Marine Chemistry,* 206: 19-33. doi: 10.1016/j.marchem.2018.08.003.

Schober, L., Dobiosova, H., Jurkas, V., Parmeggiani, F., Rudroff, F., & Winkler, M. (2023). Enzymatic reactions towards aldehydes: An overview. *Flavour and Fragrance Journal published*. 38, 221–242. doi: 10.1002/ffj.3739.

Sedjati, S., Ambariyanto, A., Trianto, A., Supriyantini, E., Ridlo, A., Bahry, M.S., Jezzi, R.R., & Sany, M.F. (2020). Antimicrobial Activity of Fungal Extract of The *Aspergillus flavus* from Hiri Island, North Maluku to Pathogenic Bacteria. *Jurnal Kelautan Tropis,* 23(1),127-135. doi: 10.14710/jkt.v23i1. 7049.

Selegato, D.M. & Castro-Gamboa, I. (2023). Enhancing chemical and biological diversity by co-cultivation. *Frontiers in Microbiology,* 14, p.1117559. doi: 10.3389/fmicb.2023.1117559.

Sun, Y., Liu, W.C., Shi, X., Zheng, H.Z., Zheng, Z.H., Lu, X.H., ... & Dong, Y.S. (2021). Inducing secondary metabolite production of Aspergillus sydowii through microbial co-culture with *Bacillus subtilis. Microbial Cell Factories*, 20(1), 1-16.

Sharma, A., Gupta, V., Khan, M., Balda, S., Gupta, N., Capalash, N., & Sharma, P. (2017). Flavonoid-rich agro-industrial residues for enhanced bacterial laccase production by submerged and solid-state fermentation. *3 Biotech, 7*, 1-8. doi: 10.1007%2Fs13205-017-0836-0.

Soler-Hurtando, M.M., Sandval-Sierra, J.V., Marchrdom, A., and Dieguez-Uribeondo, J. (2016). *Aspergillus sydowii* and Other Potential Fungal Pathogens in Gorgonian Octocorals of the Ecuadorian Pacific. *PLoS ONE,* 11(11), p.0165992. doi: 10.1371/journal.pone.0165992

Sari, D.A., Sulistyarini, I., & Rahardian, M.R.R. (2021). Anti-Bacterial Activity of Ethanol Extract, N-Hexane Fraction, Ethyl Acetate Fraction And Water Fraction From Dragon Fruit Stem (*Hylocereus Polyrhizus*) Against *Methicillin-Resistant Staphylococcus aureus* (MRSA). *Jurnal Ilmu Kesehatan* 9(2), 162–71.

Suryanarayanan, T.S. (2012). The diversity and importance of fungi associated with marine sponges. *Botanica Marina,* 55, 553-564. doi: 10.1515/bot-2011-0086.

Trianto, A., Radjasa, O.K., Subagiyo., Octaviani., E. Isabella., A.R., Bahry., M.S., Purnaweni, H., Djamaludin, R., Tjoa, A., Singleton., I., Evans, D.M., & Diele, K. (2023). Molecular Identification and Biotechnological Potential of *Cerithidea cingulate* and *Lottia scabra* Associated Fungi as Extracellular Enzyme Producer and Anti-Vibriosis Agent. *Indonesian Journal of Marine Sciences*. 28(2), 136-147. doi: 10.14710/ik.ijms.28.2.136-147.

Trianto, A., Sabdono, A., Rochaddi, B., Triingsih, D.W. (2017a). Exploration of Marine Sponges-Associated Fungi Producing Antifungal Compounds. *Asian Journal of Microbiology, Biotechnology & Environmental Sciences,* 19(3), 588-583.

Trianto, A., Widyaningsih, S., Radjasa, O.K., &Pribadi, R. (2017b). Symbiotic Fungus of Marine Sponge *Axinella* sp. Producing Antibacterial Agent. *IOP Conference Series: Earth and Environmental Science, 55*(1), p.012005. doi: 10.1088/1755-1315/55/1/012005

Walker, G.M., & White, N.A. (2005). Introduction to fungal physiology. In K. Kavanagh (Ed) *Fungi: biology and applications* (3rd ed., pp: 1-58). John Wiley & Sons. doi: 10.1002/9781119374312.ch1.

Zhu, A., Zhang, X.W., Zhang, M., Li, W., Ma, Z.Y.M., Zhu, H.J., & Cao, F. (2018). Aspergixanthones I–K, New Anti-Vibrio Prenylxanthones from the Marine-Derived Fungus *Aspergillus* sp. ZA-01. *Marine Drugs,* 16(9), 1–8. doi: 10.3390/md16090312.