Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(2): 299 – 316 (2024)
www.ejabf.journals.ekb.eg

Investigating the Biodiversity of *Clathria* sp. Sponge Symbionts from Derawan Island, Indonesia: An Exploration of Antimicrobial, Antioxidant, and Enzyme-Producing Bacteria

Sumarlin[1, 3*], Syamsidar Gaffar[2], Abu Tholib Aman[4], Respati Tri Swasono[5], Ira Maya Abdiani[1], Imra[1], Zakaria Yaran[1], Lia Fitriani[1], Elsa Kirana[1]

[1]Department of Fishery Product Technology, Fisheries and Marine Science Faculty, Universitas Borneo Tarakan, Indonesia
[2]Department of Aquatic Resources Management, Fisheries and Marine Science Faculty, Universitas Borneo Tarakan, Indonesia
[3]Research Center for Biotechnology and Biodiversity, SEATROPS, Universitas Borneo Tarakan, Indonesia
[4]Department of Clinical Microbiology, Faculty of Medicine, Public Health, and Nursing. Universitas Gadjah Mada, Indonesia
[5]Department of Chemistry, Mathematic and Natural Science Faculty, Universitas Gadjah Mada, Indonesia

[*]**Corresponding Author: sumarlin@borneo.ac.id**

ARTICLE INFO

Article History:
Received: Sept. 9, 2023
Accepted: Feb.27, 2023
Online: March 30, 2024

Keywords:
Sponge symbionts,
Derawan Island,
Antimicrobial activity,
Antioxidant potential,
Enzyme production,
Marine biotechnology

ABSTRACT

Sponges from the Clathria genus, especially those from Derawan Island, Indonesia, are renowned repositories of microbial symbionts enriched with potent bioactive compounds. Our study delved into these sponges, extracting and examining their bacterial symbionts for their antimicrobial, antioxidant, and enzymatic activities. Using dilution and streak methods, 23 distinct bacterial types were isolated and identified based on cellular morphology. Among these, the SC-06 isolate demonstrated significant antibacterial capabilities against *Staphylococcus aureus* and *Escherichia coli*. Further, isolates SC-01, SC-03, SC-11, SC-13, and SC-15 exhibited powerful antioxidant properties, neutralizing over 50% of radicals. Additionally, strains SC-08, SC-02, SC-05, and SC-22 displayed remarkable enzymatic activities, suggesting their potential industrial applications. The *Clathria* sp. sponges from Derawan offer a promising biotechnological frontier, with their bacterial symbionts poised to redefine marine biotech innovations.

INTRODUCTION

Over the past decade, the emergence of multidrug resistance (MDR) owing to microbial adaptations has positioned itself as a global challenge of unprecedented magnitude. This challenge casts its shadows over healthcare, food security, and the broader ambitions of sustainable development **(WHO, 2021; The Lancet, 2022; Larsson & Flach, 2022)**. Recognizing the pressing nature of this issue, the WHO rolled out a list in 2017 spotlighting priority MDR pathogens, underlining the critical demand for innovative antibiotics **(Asokan *et al.*, 2019; Phui-Chyng *et al.*, 2021)**. The stark reality of this looming menace is evidenced by statistics: MDR infections are currently

implicated in about 700,000 deaths each year. Without robust intervention, this figure could spiral to a staggering 10 million annually by 2050 **(Jim, 2016)**. The imperative to unearth new bioactive agents capable of subduing MDR pathogens has thus become intensely urgent **(Jakubiec-Krzesniak *et al.*, 2018)**.

While terrestrial realms have traditionally been the focal point in our search for antimicrobial substances, the vast and largely unexplored marine biodiversity presents a compelling frontier **(León-Palmero *et al.*, 2018; Stincone & Brandelli, 2020; Srinivasan *et al.*, 2021; Guo *et al.*, 2022; Krishna M. S. *et al.*, 2022)**. Within this marine expanse, sponges, especially those native to Indonesia, emerge as treasure troves of potent bioactive molecules **(Izzati *et al.*, 2021)**. Yet, direct extraction from these sponges raises sustainability and overharvesting concerns. A more sustainable approach lies in harnessing the symbiotic bacteria residing within these sponges. Remarkably, these microorganisms have the capability to produce bioactive compounds, eliminating the need to unduly exploit the sponge hosts **(Schröder *et al.*, 2003; Muller *et al.*, 2004; Dudler & Eberl, 2006; Asagabaldan *et al.*, 2017; Varijakzhan *et al.*, 2021b; Li *et al.*, 2023)**.

Situated within the Berau Regency of East Kalimantan, Indonesia, the Derawan Islands are characterized by their remarkable diversity of sponge species. This biodiversity establishes the archipelago as an invaluable natural laboratory for the exploration of symbiotic relationships between sponges and their microbial associates **(De Voogd *et al.*, 2009)**. However, the extensive bioactive potential of these marine ecosystems, particularly regarding the antibacterial, antioxidant, and enzymatic functions of bacteria associated with sponges, remains insufficiently explored.

In this regard, our research was directed at *Clathria* sp., a sponge species found in the Derawan Archipelago. The primary aim of our study was to conduct an exhaustive analysis of the antibacterial, antioxidant, and enzymatic properties of its bacterial symbionts. This investigation was intended to further elucidate the bioactive capabilities of these microorganisms, contributing to a deeper understanding of their potential applications in biotechnological and pharmaceutical domains.

MATERIALS AND METHODS

Sample collection and preservation

Our collection procedure adhered to the protocol outlined by **Asagabaldan *et al.* (2017)**. We sourced *Clathria* sp. sponges from Derawan Island, East Kalimantan, Indonesia, pinpointed at coordinates 2°16'37.1"N 118°14'39.6"E. Snorkeling at a modest depth of about 2 meters during low tide facilitated the harvest. Each gathered sponge was carefully stored in a sterile plastic bag. We placed the bags in a pre-chilled cooler box to preserve the vitality of the sponge's symbiotic bacteria and protect the samples. This cooler ensured optimal temperature during transit to the University of Borneo Tarakan's Central Laboratory of Life Sciences (LSIH). Once at the lab, we promptly stored the sponge samples in a refrigerator, maintaining a constant 4°C, awaiting further examination.

Bacterial isolation and purification process

The sponge specimen underwent the following procedure using strict aseptic techniques; it was initially cleansed with sterilized seawater. A 1cm³ section was then extracted from the sponge's core using a sanitized knife. This section was then subjected to maceration at a controlled room temperature of 24± 2°C, lasting 5 minutes. Subsequently, the sample was meticulously dissected into tiny fragments and pulverized using a clean mortar and pestle. To create serial dilutions of this sponge paste, we employed sterile filtered seawater (FS). After achieving a consistent homogenization, the concoction was amalgamated with sterilized seawater and agitated. A 1ml aliquot was drawn using a pipette and introduced to a test tube containing 9ml of sterilized seawater from this suspension. Serial dilutions ranging from 10^{-1} to 10^{-5} mL were meticulously crafted to ensure isolates were neither overcrowded nor sparse, representing the full bacterial spectrum within the sponge. From the latter dilutions (10^{-3}, 10^{-4}, 10^{-5}), 100µL samples were spread across marine agar plates. To safeguard against fungal invasion, we incorporated nystatin (50mg/ ml) as recommended by **Nofiani *et al.* (2020)** into the culture medium. These plates were then placed in an incubation environment for a duration of 5- 7 days at a temperature of 30± 2°C, as suggested by **Bibi *et al.* (2021)**. Lastly, to achieve pure bacterial cultures, individual colonies, differentiated by their morphological attributes, were singled out and transitioned to fresh media using the streak technique, in line with the method described by **Asagabaldan *et al.* (2017)**.

Antimicrobial activity screening against multidrug-resistant bacterial pathogens

We screened the antimicrobial potential of sponge-associated bacteria by challenging them against multidrug-resistant (MDR) isolates of *S. aureus* ATCC 25923 and *E. coli* ATCC 11229. These isolates were sourced from the Clinical Microbiology Laboratory at the Faculty of Medicine, Gadjah Mada University, Yogyakarta. For this endeavour, we employed the overlay method as described by **Asagabaldan *et al.* (2017)**. Each bacterial isolate, after rejuvenation, was sampled with a 10µL aliquot and introduced into 6mm wells crafted in agar media. This setup was incubated at a specific temperature conducive to bacterial growth (30± 2°C) over a span of 18- 20 hours. Concurrently, the MDR pathogenic bacteria were cultivated in a semi-solid agar medium (specifically, Muller Hinton fortified with 50% agar) calibrated to a 0.5 McFarland concentration. This was subsequently layered atop the previously incubated bacterial isolate. Following the layering, the medium was subjected to the ideal growth temperature of the pathogenic bacteria, set at 37± 2°C, and maintained for 24 hours. The efficacy against MDR pathogens was gauged by the presence of an inhibition diameter (ID) encircling the bacterial isolate. For reference, ampicillin at a concentration of 100ppm served as the positive control, while dimethyl sulfoxide (DMSO) was employed as the negative control. All experiments were reiterated thrice for consistency.

Evaluation of bacterial antioxidant capabilities

We assessed the antioxidant potential of bacterial isolates, anchoring our methodology on **Sulistiyani *et al.* (2016)** and **Kim *et al.* (2022)**, albeit with nuanced adjustments. To induce the production of secondary metabolites, the bacterial isolates were cultured in marine broth medium and stirred at 180rpm on a rotary incubator shaker for a duration of 7 days. A subsequent centrifugation step at 10,000rpm for 10 minutes at

4°C ensured the retrieval of cell-free supernatant, which was then subjected to the DPPH free radical scavenging assay. For the assay, 0.5mL of the supernatant was combined with 3mL of a 0.1mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in methanol. An additional 1.5mL of methanol was integrated to achieve a total volume of 5mL. The control for the assay utilized sterile marine broth in lieu of the sample supernatant, while methanol was the designated blank. The ideal absorbance for DPPH was ascertained across a wavelength spectrum spanning 200- 700nm. A benchmark positive control was set using ascorbic acid. The efficacy of the radical scavenging was deduced via the equation:

$$\%\ Scavenging\ activity = \frac{A_o - A_1}{A_n}\ x\ 100$$

Where, A_o stands for the absorbance of DPPH coupled with the blank (Methanol),
A_1 represents the absorbance of DPPH when integrated with the supernatant (Sample).

To ensure reliability, all experimental setups were executed three times. The resultant data were articulated as an average paired with its standard deviation (SD). The significance of disparities between samples was appraised using one-way ANOVA on SPSS (Version 25.0, SPSS Inc., Chicago, IL), supplemented by the Tukey-honestly significant difference test. Statistical significance was adjudged at a threshold of $P< 0.05$, echoing the stipulations of **Xing *et al.* (2015)** and **Sulistiyani *et al.* (2016)**.

Screening of bacteria for enzymatic activities

In the quest to uncover enzymatic capabilities, substrates at a concentration of 1% – skimmed milk, amylum, tributyrin, and CMC (carboxymethyl cellulose) – were judiciously employed. These substrates were meticulously infused into Zobel marine agar medium. Following this, the prospective symbiotic bacterial strains were evaluated for their prowess in generating enzymes, namely protease, amylase, lipase, and cellulase, utilizing the 6mm well method **(Bibi *et al.*, 2018, 2020)**. Remarkably, the emergence of distinct zones encircling the bacterial colonies signified the presence of proteolytic and lipolytic activities. For gauging amylolytic and cellulolytic capacities, an iodine solution was incorporated and allowed to interact for a span of about 5 minutes. A pronounced clear zone encircling these colonies was an unequivocal indication of robust amylase and cellulase enzymatic production **(Ahmad *et al.*, 2013; Viswanathan & Rebecca, 2019; Bibi *et al.*, 2020; Maharsiwi *et al.*, 2020)**. The enzymatic index was calculated using the appropriate formula **(Maharsiwi *et al.*, 2020)**:

$$Enzymatic\ index = \frac{diameter\ of\ zone - colony\ diameter}{colony\ diameter}$$

Enzymatic activities were systematically analyzed, with findings presented as the mean ± standard deviation (SD) based on results from three distinct experiments. For in-depth statistical interpretations, we employed the SPSS software (Version 25.0, SPSS Inc., Chicago, IL). The data set's distribution was evaluated using the one-way analysis of variance (ANOVA), which was sequentially complemented by Tukey's honestly significant difference test. This analytical approach was chosen to discern noteworthy variations in the enzymatic activity metrics across the diverse bacterial isolates studied.

The threshold for discerning statistical significance was determined as a *P*-value of less than 0.05 *(P*< 0.05).

RESULTS AND DISCUSSION

Our study successfully isolated bacteria associated with *Clathria* sponges from Derawan Island's waters in Berau Regency, Indonesia. To determine the exact taxonomy of the sponges collected, we cross-referenced our samples with entries in the iNaturalist database, accessible at https://inaturalist.nz/taxa/63671-Clathria. Notably, our findings are congruent with the descriptions of *Clathria* sp. recorded in the database.

Further analysis confirmed our sponge samples as *Clathria* sp., a member of the distinguished Microcionidae family, as illustrated in Fig. (1). Interestingly, *Clathria* sp. sponges are part of the Demospongiae class. Members of this class are unique marine organisms without skeletons, recognized for their abundant bioactive compounds. The potential of these compounds underscores the significance of Demospongiae in the pioneering realm of pharmaceutical biotechnology.

Fig. 1. Illustration of *Clathria* sp. from the Microcionidae family isolated from the waters of Derawan Island

Isolation and characterization of symbiotic bacteria

The culmination of our investigative effort was the efficacious isolation of 23 unique bacterial strains associated symbiotically with marine sponges, as detailed in Table (1). The classification process for these isolates revolved around an exhaustive analysis of their colony morphological features. The evaluation considered a variety of characteristics, from colony form and edge definition to color nuances and elevational aspects. Subsequent Gram staining examinations divulged that a significant proportion of these isolates conformed to the traits of Gram-positive bacteria, most notably assuming a coccus cellular form.

The spectrum of bacterial strains identified in our research underscores the deep-seated and multifaceted microbial symbiosis prevalent within sponges. It is of particular

interest that this inherent microbial assemblage accounts for an impressive 40% of a sponge's aggregate biomass. This fact emphasizes the symbiotic bond shared between the sponge and its embedded microorganisms. Our observations align harmoniously with those presented by **Bibi *et al.* (2020)**. Their study, which probed the symbiotic bacterial community associated with sponges, also utilized diverse culturing mediums, including marine agar—an essential component of our investigative procedure. Our findings bolster the existing scholarly perspective: marine sponges, abundant with a myriad of symbiotic bacterial species, emerge as a valuable resource for cutting-edge endeavours in biotechnological and medical domains.

Tabel 1. Morphological characteristics of *Clathria* sp. sponge symbiotic bacteria

Isolate Id	Observation Result					
	Color	Margin	Forms	Elevation	Gram stain	Cell shape
SC-01	Yellow	Irregular	Round with scalloped margin	Flat	-	Coccus
SC-02	Yellow	Lobate	Round with scalloped margin	Raised	+	Coccus
SC-03	White	Undulate	Irreguler and spreading	Raised	+	Coccus
SC-04	Yellow	Entire	Round with raised margin	Raised	-	Coccus
SC-05	White	Undulate	Irreguler and spreading	Flat	+	Bacillus
SC-06	Yellow	Undulate	Round with scalloped margin	Flat	+	Coccus
SC-07	Yellow	Undulate	Concentric	Raised	+	Bacillus
SC-08	Yellow	Undulate	L-Form	Umbonate	+	Coccus
SC-09	Yellow	Undulate	Irreguler and spreading	Flat	+	Coccus
SC-10	White	Lobate	Irreguler and spreading	Flat	+	Coccus
SC-11	White	Lobate	Round with raised margin	Flat	+	Coccus
SC-12	White	Undulate	Round with scalloped margin	Raised	+	Coccus
SC-13	Yellow	Irregular	Wrinklead	Raised	+	Coccus
SC-14	White	Lobate	L-Form	Umbonate	+	Coccus
SC-15	Yellow	Irregular	Round with scalloped margin	Raised	+	Coccus
SC-16	White	Lobate	Wrinklead	Flat	-	Coccus
SC-17	White	Entire	Round with scalloped margin	Flat	+	Bacillus
SC-18	White	Irregular	Rhizoid	Raised	+	Coccus
SC-19	Yellow	Undulate	Round with raised margin	Flat	+	Coccus
SC-20	White	Irregular	Round with scalloped margin	Flat	+	Coccus
SC-21	White	Entire	Round with raised margin	Raised	+	Coccus
SC-22	White	Undulate	Concentric	Raised	+	Coccus
SC-23	White	Undulate	Round with raised margin	Flat	-	Coccus

Screening for antibacterial activities against MDR pathogen bacteria

This investigation probed the antimicrobial prowess of bacteria intricately symbiotic with marine sponges, targeting two predominant human pathogens: the Gram-positive bacterium, *Staphylococcus aureus*, and the Gram-negative bacterium, *Escherichia coli*. Our choice of these microorganisms was underpinned by their significant clinical implications and distinguished standing as referential organisms in antibacterial studies.

From the preliminary assays, eight bacterial isolates emerged with demonstrable antimicrobial efficacy. These isolates, enumerated in Table (2), encompass SC- 01, SC- 02, SC- 03, SC- 06, SC- 10, SC- 12, SC- 19, and SC- 20. Intriguingly, isolates SC- 01 and SC- 06 exhibited the capacity to inhibit both bacterial categories. In contrast, while isolates SC- 03, SC- 19, and SC- 20 showcased a particular predilection for thwarting *S. aureus*, isolates SC- 02, SC- 10, and SC- 12 manifested pronounced inhibitory effects against *E. coli*.

To provide a granular characterization of the antimicrobial influence, we formulated an evaluative scale predicated on the extent of the inhibitory zones' diameter. Adhering to benchmarks delineated by **Cita *et al.* (2017)**, isolates producing inhibition zones exceeding 16.0 mm were classified as demonstrating 'strong' activity. Those yielding zones spanning 11- 16mm were categorized as 'moderate', the 7- 11mm range was adjudged 'weak', and zones under 7mm were discerned as devoid of antimicrobial prowess. A salient observation surfaced with isolate SC- 06, which registered an exceptionally expansive inhibition zone, amplifying its potential as a formidable antibacterial agent.

Tabel 2. Antagonistic activity screening from bacteria symbionts *Clathria* sp.

Lab number	**Clear zone (mm)**		**Growth inhibition response**
	S.aureus	***E.coli***	
SC-01	13.5 ± 0.70	15 ± 2.82	Strong
SC-02	-	14.5 ± 2.12	Strong
SC-03	10.5 ±0.70	-	Strong
SC-06	21.5 ± 2.12	15.5 ± 2.12	Strong
SC-10	-	15	Strong
SC-12	-	14	Strong
SC-19	10.5 ± 0,70	-	Strong
SC-20	15 ± 1,41	-	Strong
Positive Control	20	17	Strong
Negative Control	-		

Marine sponges, integral components of oceanic ecosystems, provide shelter to a rich assemblage of microbial entities, collectively termed as the 'microbiome'. This intricate association transcends a mere cohabitation; the microbiome plays a crucial role in fortifying the host's nutritional and defensive capabilities **(Anteneh *et al.*, 2021; Díez-Vives *et al.*, 2022)**. In this symbiotic nexus, both the sponge and its microbial denizens contribute to the mutualistic relationship, ensuring reciprocal benefits and evolutionary resilience.

The antimicrobial activity demonstrated by the bacteria associated with sponges has garnered significant academic attention. Their capacity to thwart the proliferation of pathogenic bacterial strains offers promising avenues for scientific investigation. It is plausible that the continual interactions within this confined microenvironment have engendered a sophisticated repertoire of defensive compounds, conferring an evolutionary advantage to the symbionts. The purpose of these bioactive compounds is to protect the symbiotic bacteria from potential pathogens and marine predators.

Highlighting the profundity of this association, numerous novel bioactive compounds with potential therapeutic implications have been identified, with their origins traced back to the microbial symbionts residing within sponges **(Anteneh *et al.*, 2021)**. This underscores the importance of delving deeper into these symbiotic dynamics, not merely for ecological comprehension but as a promising frontier for pharmaceutical research and biotechnological advancements. Hence, the academic community is beckoned to intensify their research endeavours, unraveling the myriad antimicrobial secrets nestled within these marine sponges and their resident microbiomes.

Clathria species have been illuminated in scientific literature for their prodigious capacity to produce compounds exhibiting a spectrum of bioactivities. These compounds have been ascertained to possess antibacterial, antiviral, and even antifungal properties **(Rudi *et al.*, 2001; Cheung *et al.*, 2014; El-Demerdash *et al.*, 2018; Kibungu *et al.*, 2021)**. One particularly intriguing discovery is that of clathsterol, a unique sterol sulfate. **Rudi *et al.* (2001)** isolated this compound from a *Clathria* species, establishing its notable efficacy against HIV-1 RT, a pivotal enzyme in the life cycle of the HIV virus. Such findings underscore the immense potential that marine sponges, and specifically the *Clathria* species, harbor in the realm of antiviral research.

Antioxidant activity of sponge-associated bacteria

In the intricate domain of marine biology, marine sponges stand out as pivotal sanctuaries for symbiotic microorganisms. These microorganisms present profound biotechnological potential, primarily attributed to their ability to synthesize antioxidant compounds. Fig. (2), delineated in this research, delves deeply into the antioxidant proficiencies of these bacteria allied with marine sponges, highlighting their adeptness at quenching DPPH radicals.

Upon scrutinizing the gathered data, we discerned marked variations in antioxidant efficiencies among the bacterial isolates studied. While some isolates manifested a relatively moderate efficiency, hovering around 27%, others exhibited a remarkable efficiency, soaring to 56%. Such variations accentuate the unique attributes inherent to each bacterial isolate, reinforcing their pivotal contributions to antioxidant processes.

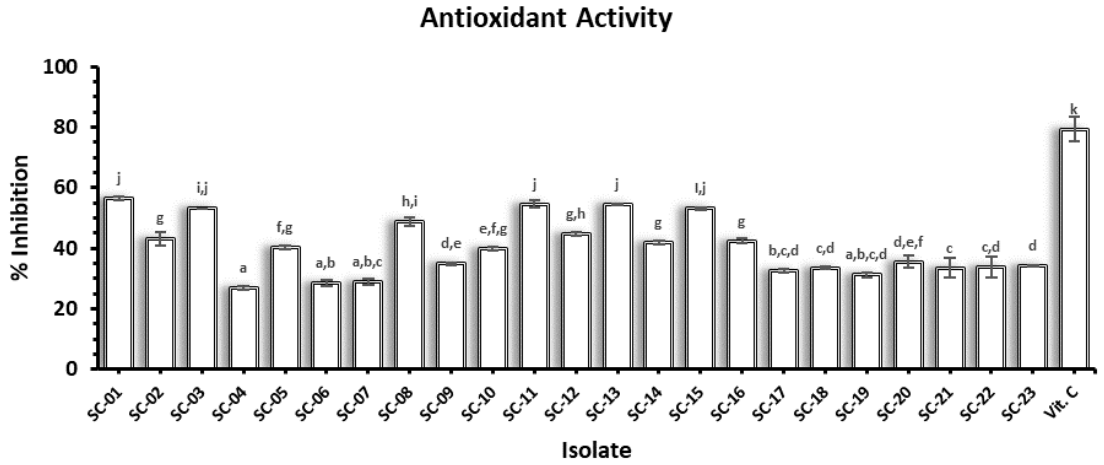

Fig. 2. Activity of DPPH free radical scavenging. A post-hoc analysis was employed utilizing the Tukey-HSD method, with a significance threshold set at $P< 0.05$. Distinct superscripts denote statistically significant differences.

At their core, antioxidants are molecules tailored to mitigate or impede cellular damage induced by free radicals. These radicals surface during metabolic activities such as digestion or upon exposure to adverse factors viz. radiation and tobacco. Importantly, radicals, including the reactive oxygen species (ROS), precipitate oxidative stress, a foundational element for a plethora of diseases and age-related disorders. Hence, compounds displaying antioxidant properties, such as those found in sponge-associated

bacteria, hold considerable therapeutic prospects **(Brinkmann *et al.*, 2017; Esposito *et al.*, 2021; Carrier *et al.*, 2022)**.

Enzymatic activities of antagonistic bacteria production

The marine environment, particularly sponge symbionts, represents an uncharted treasure trove of potential bioactive compounds and enzymatic activities **(Varijakzhan *et al.*, 2021a)**. The *Clathria* sp. sponge, indigenous to the waters of Pulau Derawan, hosts myriad bacterial symbionts. In this study, we managed to isolate 23 distinct bacterial strains from the *Clathria* sp. sponge and further delved into their potential enzymatic capabilities, focusing on proteolytic, amylolytic, lipolytic, and cellulolytic activities (Table 3). Based on the transformation of the findings of previous studies **(Ahmad *et al.*, 2013; Cheng *et al.*, 2020)**, we established an interpretation index for enzymatic activity. Values below 0.8 are considered weak, values between 0.8 and 2.4 are moderate, and values exceeding 2.5 are strong. Our investigation revealed that the symbiotic bacterial isolates exhibited moderate enzymatic activity, except for lipolytic enzymes, which were classified as strong.

Proteolytic activity

Proteolytic enzymes have a wide range of industrial applications, including in the food industry for protein breakdown during fermentation and ripening processes, in the textile industry for fabric bleaching and pre-washing, and in the medical field for various therapies and treatments **(Queiroga *et al.*, 2012; Raveendran *et al.*, 2018; Tavano *et al.*, 2018; Razzaq *et al.*, 2019; Solanki *et al.*, 2021)**. In particular, proteases are one of the most widely used industrial enzymes with tremendous applications in various industries including detergent, leather, silver recovery, dairy, baking, beverages, and pharmaceutical industries. Researchers are exploring various approaches to discover, redesign, or artificially synthesize enzymes with better applicability in industrial processes. These enzymes offer a sustainable and environmentally safer option in addition to possessing an economic and a commercial value **(Solanki *et al.*, 2021)**.

The outcomes of the study indicate that several isolates exhibited a commendable capacity to hydrolyze proteins. Specifically, the isolates SC- 06, SC- 08, and SC- 19 demonstrated pronounced proteolytic efficiency. The enzymatic metrics for these strains were 1.34± 0.09, 1.34± 0.10, and 1.33± 0.08, respectively. The data gleaned from this research substantiates the premise that marine bacteria possess the capability to synthesize proteases. The marked proteolytic performance by SC- 06, SC- 08, and SC- 19 underscores the potential of these bacterial strains in spearheading novel biotechnological innovations aimed at protein decomposition.

Collectively, the insights from these investigations support the proposition that marine bacteria hold significant promise as a reservoir for biotechnological advances targeting protein degradation. Given that proteins form the bedrock of both animal and plant cellular structures, harnessing their degradation potential paves the way for the synthesis of food enhancers, pharmaceutical formulations, and an array of value-added products.

Tabel 3. Enzymatic activities of bacteria symbionts *Clathria* sp.

Isolate code	Enzyme activity					
	Protease			Cellulase		
	DZ	DC	EI	DZ	DC	EI
SC-01	30.33 ± 0.57	18 ± 1.0	0.68 ± 0.09^{a,b}	42 ± 1.73	24.66 ± 0.57	0.70 ± 0.03^{c,d,e}
SC-02	7	7	0	8	8	0
SC-03	30 ± 2.0	15.66 ± 0.57	0.92 ± 0.07^{a,b,c,d,e}	41.33 ± 1.52	26 ± 1.0	0.59 ± 0.12^{b,c,d,e}
SC-04	19.66 ± 1.52	8 ± 1.0	1.5 ± 0.44^{f,g,h}	10	10	0
SC-05	18.33 ± 1.15	9.66 ± 0.57	0.9 ± 0.2^{a,b,c,d,e}	36.33 ± 1.52	17.66 ± 0.57	1.06 ± 0.16^{g}
SC-06	30.33 ± 0.57	11.66 ± 0.57	1.6 ± 0.11^{g,h}	39.33 ± 1.15	21.66 ± 1.52	0.82 ± 0.15^{e,f,g}
SC-07	29 ± 1.0	12.33 ± 0.57	1.35 ± 0.18^{e,f,g,h}	29.33 ± 1.15	17.33 ± 0.57	0.69 ± 0.11^{c,d,e}
SC-08	24.66 ± 0.57	9 ± 1.0	1.76 ± 0.25^{h}	15.33 ± 0.57	9.66 ± 0.57	0.59 ± 0.08^{b,c,d,e}
SC-09	31 ± 1.0	15.66 ± 0.57	0.98 ± 0.03^{a,b,c,d,e}	20	20	0
SC-10	10	10	0	14.66 ± 0.57	11.66 ± 0.57	0.26 ± 0.01^{a}
SC-11	27 ± 1.0	17.66 ± 0.57	0.53 ± 0.03^{a}	43.66 ± 1.52	28.33 ± 0.57	0.54 ± 0.06^{a,b,c,d,e}
SC-12	30.66 ± 0.57	15.33 ± 0.57	1.01 ± 0.1^{a,b,c,d,e,f}	43 ± 1.73	28.66 ± 0.57	0.5 ± 0.05^{a,b,c,d}
SC-13	30.66 ± 0.57	15.33 ± 0.57	1.01 ± 0.1^{a,b,c,d,e,f}	29	29	0
SC-14	28 ± 1.0	15 ± 1.0	0.87 ± 0.06^{a,b,c,d,e}	45.33 ± 0.57	31.66 ± 1.52	0.43 ± 0.08^{a,b,c}
SC-15	19 ± 1.0	10.33 ± 0.57	0.84 ± 0.05^{a,b,c,d}	12	12	0
SC-16	28.66 ± 0.57	12.66 ± 0.57	1.26 ± 0.06^{c,d,e,f,g}	41 ± 1.0	20.33 ± 0.57	1.02 ± 0.1^{f,g}
SC-17	28.66 ± 0.57	15.66 ± 0.57	0.83 ± 0.09^{a,b,c,d}	45 ± 1.0	32.66 ± 0.57	0.37 ± 0.04^{a,b}
SC-18	23.66 ± 1.52	13 ± 1.0	0.82 ± 0.03^{a,b,c}	19	19	0
SC-19	28 ± 1.0	10.66 ± 0.57	1.63 ± 0.23^{g,h}	43.66 ± 1.15	18.33 ± 1.53	1.39 ± 0.14^{h}
SC-20	27.33 ± 1.52	11.66 ± 0.57	1.34 ± 0.08^{e,f,g,h}	31 ± 1.0	17.66 ± 1.53	0.76 ± 0.18^{d,e,f}
SC-21	25.66 ± 0.57	12.66 ± 0.57	1.03 ± 0.12^{b,c,d,e,f}	35.66 ± 1.52	17.66 ± 1.15	1.02 ± 0.14^{f,g}
SC-22	23 ± 1.0	12.66 ± 0.57	0.82 ± 0.04^{a,b,c}	32 ± 2.0	10.66 ± 0.57	2.0 ± 0.09^{i}
SC-23	26 ± 1.0	11.33 ± 1.53	1.32 ± 0.27^{d,e,f,g,h}	15	15	0
	Lipase			Amilase		
	DZ	DC	EI	DZ	DC	EI
SC-01	23.33 ± 0.57	20 ± 1.0	0.16 ± 0.04^{a}	24.33 ± 0.57	18 ± 1.0	0.35 ± 0.05^{a,b,c}
SC-02	31 ± 1.0	7.33 ± 0.57	3.25 ± 0.43^{e}	6	6	0
SC-03	22	22	0	22.66 ± 1.52	18.33 ± 0.57	0.24 ± 0.06^{a,b}
SC-04	14 ± 1.0	6.66 ± 0.57	1.10 ± 0.09^{c}	21.66 ± 1.52	10.33 ± 0.57	1.11 ± 0.25^{d}
SC-05	13.66 ± 1.52	7.33 ± 0.57	0.86 ± 0.23^{b,c}	19.66 ± 1.52	9.33 ± 1.15	1.14 ± 0.43^{d}
SC-06	19 ± 1.0	14 ± 1.0	0.36 ± 0.08^{a}	22.33 ± 2.08	15.66 ± 1.52	0.43 ± 0.19^{a,b,c}
SC-07	14.33 ± 1.52	10 ± 1.0	0.43 ± 0.02^{a,b}	19.66 ± 1.52	12.33 ± 0.57	0.59 ± 0.13^{b,c}
SC-08	15 ± 1.0	11.66 ± 0.57	0.28 ± 0.04^{a}	13 ± 1.0	9 ± 1.0	0.44 ± 0.05^{a,b,c}
SC-09	18 ± 1.0	15.66 ± 0.57	0.15 ± 0.1^{a}	19 ± 1.0	13 ± 1.0	0.46 ± 0.03^{a,b,c}
SC-10	22 ± 2.0	7.33 ± 0.57	2.02 ± 0.47^{d}	6	6	0
SC-11	27	27	0	24.33 ± 1.15	17.66 ± 0.57	0.38 ± 0.02^{a,b,c}
SC-12	17 ± 1.0	16.33 ± 0.57	0.04 ± 0.07^{a}	23 ± 2.64	17.66 ± 0.57	0.30 ± 0.17^{a,b}
SC-13	18	18	0	22 ± 2.0	18 ± 1.0	0.23 ± 0.16^{a,b}
SC-14	19.33 ± 0.57	17.33 ± 0.57	0.12 ± 0.06^{a}	22.66 ± 0.57	19.33 ± 0.57	0.17 ± 0.03^{a,b}
SC-15	16	16	0	11	11	0
SC-16	18.33 ± 0.57	16.33 ± 0.57	0.12 ± 0.01^{a}	21.33 ± 1.52	14 ± 1.0	0.53 ± 0.21^{b,c}
SC-17	17	17	0	23.66 ± 0.57	20.33 ± 0.57	0.16 ± 0.03^{a,b}
SC-18	17	15.66 ± 0.57	0.08 ± 0.04^{a}	21.33 ± 1.15	17.66 ± 0.57	0.21 ± 0.02^{a,b}
SC-19	19	19	0	18 ± 1.0	13.33 ± 0.57	0.35 ± 0.13^{a,b,c}
SC-20	16.33 ± 0.57	14.33 ± 0.57	0.13 ± 0.01^{a}	23 ± 1.0	13 ± 1.0	0.78 ± 0.18^{c,d}
SC-21	20	20	0	20 ± 1.0	16.66 ± 0.57	0.20 ± 0.09^{a,b}
SC-22	16.66 ± 0.57	13.66 ± 0.57	0.22 ± 0.01^{a}	20.33 ± 1.52	13 ± 1.0	0.56 ± 0.02^{b,c}
SC-23	17.33 ± 0.57	12.66 ± 0.57	0.37 ± 0.05^{a}	18.33 ± 1.52	11.33 ± 0.57	0.63 ±0.2^{b,c}

*Post-hoc analysis was performed using the Tukey-HSD method with a significance level of $P< 0.05$. Different superscripts in the same column indicate significant differences.

Cellulolytic activity

Cellulose, an integral component of plant cell walls, reigns as the planet's most prolific organic compound. Structurally, it comprises a linear array of glucose units, interconnected by beta-1,4 glycosidic linkages. Due to its stability, cellulose presents a formidable challenge to degradation processes.

In our recent investigation, we meticulously selected bacterial isolates based on their potential to break down cellulose. These isolates were cultivated in a specialized medium where cellulose was the exclusive carbon provider. The degradation efficacy was quantified by assessing the liberated reducing sugars **(P. Gupta *et al.*, 2012; Zhang & Dong, 2022)**.

Our data revealed that multiple isolates exhibited a commendable capability for cellulose degradation. Remarkably, isolate SC- 22 stood out, registering an enzymatic index of 2± 0.09. This research underscores the potential of marine bacteria in producing cellulases - specialized enzymes adept at cellulose degradation. SC- 22, with its elevated cellulolytic prowess, bears significant promise for spearheading innovative biotechnological endeavours centered on cellulose degradation.

Collectively, such revelations accentuate the untapped reservoir of marine bacteria as pivotal catalysts in contemporary biotechnological pursuits related to cellulose decomposition. Given that cellulose dominates plant biomass—a prospective wellspring of renewable energy—its efficient degradation could pave the way for biofuel production, including ethanol and biogas. Beyond energy, cellulases also hold potential in the manufacturing of diverse products, ranging from animal feed to pharmaceutical derivatives **(John *et al.*, 2022)**.

Lipolytic activity

Lipases are pivotal enzymes that orchestrate the hydrolysis of lipids, encompassing fats and oils. Their roles are multifaceted, spanning from essential biological operations, such as digestion, metabolism, and cellular signaling to commercial exploits in biodiesel synthesis, food processing, and detergent formulation.

In our investigative pursuit, we meticulously examined specific bacterial isolates for their lipolytic potential. These isolates underwent cultivation in a specialized medium with olive oil as the singular carbon constituent. The efficacy of lipid hydrolysis was gauged by quantifying the liberated fatty acids **(Patel *et al.*, 2019; Priyanka *et al.*, 2019; Mehta *et al.*, 2021)**.

Our empirical findings spotlighted two prominent isolates, SC- 02 and SC- 10, displaying remarkable lipolytic prowess. The enzymatic indices were revealing: 3.25± 0.43 for SC- 02 and 2.02± 0.47 for SC- 10. It intimates that marine bacteria might emerge as a coveted reservoir of industrially relevant lipases. Intriguingly, lipases extracted from marine bacterial matrices have demonstrated superior stability and heightened activity compared to their counterparts derived from terrestrial flora and fauna. Such observations are in harmony with previous scholarly endeavours.

The prospective avenues for marine bacterial lipases are extensive. These enzymes could catalyze the synthesis of biodiesel—a sustainable energy derivative derived from either vegetable oils or animal fats. Additionally, they harbor potential in refining the organoleptic properties of foodstuffs, as well as in the remediation of undesired fats and oils from industrial effluents **(Patel *et al.*, 2019)**.

Amylolitic activity

Amylase, a quintessential enzyme, is responsible for facilitating the hydrolysis of starch— a sophisticated carbohydrate abundantly present in plant structures. Upon its action, starch undergoes decomposition into more rudimentary molecules, notably glucose, serving as instrumental energy sources. Beyond its biological significance in processes such as digestion, metabolic regulation, and cellular communication, amylase also has pivotal roles in diverse industrial contexts, encompassing food refinement, biofuel synthesis, and the textile industry.

In the confines of this research, we meticulously screened a curated assembly of bacterial strains for their amylolytic capabilities. These isolates were cultured on a medium where starch was designated as the primary carbon donor. The proficiency of starch hydrolysis was gauged by quantifying the resultant reducing sugars **(R. Gupta *et al.*, 2003; Souza & Magalhães, 2010; Gopinath *et al.*, 2017)**.

Our findings delineate that an overwhelming proportion of the isolates exhibited pronounced amylolytic characteristics. Specifically, the strain SC- 05 manifested the most robust amylolytic propensity, registering an enzymatic index of 1.14± 0.43. Meanwhile, the strain SC- 04 demonstrated an almost analogous amylolytic prowess, with an index of 1.13± 0.37.

Such revelations bear profound implications, strongly positing marine bacteria as potential reservoirs for industrially pertinent amylases. It's noteworthy to mention that, marine-derived amylases have empirically displayed enhanced stability and catalytic vigor compared to their counterparts from terrestrial sources, including flora and fauna. Envisaging the potential industrial spectrum for marine-sourced amylases, their applications appear multifaceted. These enzymatic catalysts can be ingeniously employed to refine the organoleptic properties of food, pioneer biofuel generation, and adeptly expunge starch residues from industrial effluents **(Suriya *et al.*, 2016; Goel *et al.*, 2022)**.

CONCLUSION

The intricate biodiversity of bacterial symbionts within the Clathria genus sponges from Derawan Island, Indonesia, has unveiled a profound biotechnological reservoir nestled within the marine realm. These sponges, emblematic of nature's intricate design, harbor a plethora of microbial partners teeming with potent bioactive constituents. Several of the 23 meticulously isolated bacterial phenotypes, distinguished by their cellular architecture, have manifested pronounced bioactive proclivities. Notably, the SC-06 isolate has exhibited formidable antibacterial prowess against pathogens such as *Staphylococcus aureus* and *Escherichia coli*. Concurrently, isolates SC- 01, SC- 03, SC-11, SC- 13, and SC- 15 have showcased robust antioxidative capacities, neutralizing an excess of 50% of radical entities. A select cohort, including strains SC- 08, SC- 02, SC-05, and SC- 22, has also evinced commendable enzymatic functionalities, hinting at their prospective industrial ramifications. The *Clathria* sp. sponges from Derawan Island epitomize a promising biotechnological frontier, with their bacterial symbionts poised to sculpt the contours of marine biotechnological advancements.

ACKNOWLEDGMENTS

We would like to express our gratitude to the Ministry of Education, Culture, Research and Technology of the Republic of Indonesia for the indispensable financial support given to us under decision number 0267/E5/AK.04/2022 and contract number 005/UN51.9/SP2H-LT/2022. This funding played a pivotal role in enabling us to accomplish our research objectives at every phase of our study.

REFERENCES

Ahmad, B.; Nigar, S.; Sadaf Ali Shah, S.; Bashir, S.; Ali, J.; Yousaf, S. and Bangash, J. A. (2013). Isolation and Identification of Cellulose Degrading Bacteria from Municipal Waste and Their Screening for Potential Antimicrobial Activity. *World Applied Sciences Journal*, *27*(11): 1420–1426. https://doi.org/10.5829/idosi.wasj.2013.27.11.81162

Anteneh, Y. S.; Yang, Q.; Brown, M. H. and Franco, C. M. M. (2021). Antimicrobial Activities of Marine Sponge-Associated Bacteria. *Microorganisms*. https://doi.org/10.3390/microorganisms9010171

Asagabaldan, M. A.; Ayuningrum, D.; Kristiana, R.; Sabdono, A.; Radjasa, O. K. and Trianto, A. (2017). Identification and Antibacterial Activity of Bacteria Isolated from Marine Sponge Haliclona (Reniera) sp. against Multi-Drug Resistant Human Pathogen. *IOP Conference Series: Earth and Environmental Science*, *55*: 012019. https://doi.org/10.1088/1755-1315/55/1/012019

Asokan, G. V, Ramadhan, T.; Ahmed, E. and Sanad, H. (2019). WHO Global Priority Pathogens List: A Bibliometric Analysis of Medline-PubMed for Knowledge Mobilization to Infection Prevention and Control Practices in Bahrain. *Oman Medical Journal*, *34*(3): 184–193. https://doi.org/10.5001/omj.2019.37

Bibi, F.; Naseer, M. I. and Azhar, E. I. (2021). Assessing the diversity of bacterial communities from marine sponges and their bioactive compounds. *Saudi Journal of Biological Sciences*, *28*(5): 2747–2754. https://doi.org/10.1016/j.sjbs.2021.03.042

Bibi, F.; Strobel, G. A.; Naseer, M. I.; Yasir, M.; Khalaf Al-Ghamdi, A. A. and Azhar, E. I. (2018). Microbial Flora Associated with the Halophyte–Salsola imbricate and Its Biotechnical Potential. *Frontiers in Microbiology*, *9*(65): 1–12. https://doi.org/10.3389/fmicb.2018.00065

Bibi, F.; Yasir, M.; Al-Sofyani, A.; Naseer, M. I. and Azhar, E. I. (2020). Antimicrobial activity of bacteria from marine sponge Suberea mollis and bioactive metabolites of Vibrio sp. EA348. *Saudi Journal of Biological Sciences*, *27*(4): 1139–1147. https://doi.org/10.1016/j.sjbs.2020.02.002

Brinkmann, C. M.; Marker, A. and Kurtböke, D. I. (2017). An Overview on Marine Sponge-Symbiotic Bacteria as Unexhausted Sources for Natural Product Discovery.

In *Diversity*. https://doi.org/10.3390/d9040040

Carrier, T. J.; Maldonado, M.; Schmittmann, L.; Pita, L.; Bosch, T. C. G. and Hentschel, U. (2022). Symbiont Transmission in Marine Sponges: Reproduction, Development, and Metamorphosis. In *BMC Biology*. https://doi.org/10.1186/s12915-022-01291-6

Cheng, T. H.; Ismail, N.; Kamaruding, N.; Saidin, J. and Danish-Daniel, M. (2020). Industrial enzymes-producing marine bacteria from marine resources. *Biotechnology Reports*, *27*: 1–9. https://doi.org/10.1016/j.btre.2020.e00482

Cheung, R. C. F.; Wong, J. H.; Pan, W. L.; Chan, Y. S.; Yin, C. M.; Dan, X. L.; Wang, H. X.; Fang, E. F.; Lam, S. K.; Ngai, P. H. K.; Xia, L. X.; Liu, F.; Ye, X. Y.; Zhang, G. Q.; Liu, Q. H.; Sha, O.; Lin, P.; Ki, C.; Bekhit, A. A.; … Ng, T. B. (2014). Antifungal and Antiviral Products of Marine Organisms. In *Applied Microbiology and Biotechnology*. https://doi.org/10.1007/s00253-014-5575-0

Cita, Y. P.; Suhermanto, A.; Radjasa, O. K. and Sudharmono, P. (2017). Antibacterial activity of marine bacteria isolated from sponge Xestospongia testudinaria from Sorong, Papua. *Asian Pacific Journal of Tropical Biomedicine*, *7*(5): 450–454. https://doi.org/10.1016/j.apjtb.2017.01.024

De Voogd, N. J.; Becking, L. E. and Cleary, D. F. R. (2009). Sponge community composition in the Derawan Islands, NE Kalimantan, Indonesia. *Marine Ecology Progress Series*, *396*(12): 169–180. https://doi.org/10.3354/meps08349

Díez-Vives, C.; Koutsouveli, V.; Conejero, M. and Riesgo, A. (2022). Global Patterns in Symbiont Selection and Transmission Strategies in Sponges. In *Frontiers in Ecology and Evolution*. https://doi.org/10.3389/fevo.2022.1015592

Dudler, R. and Eberl, L. (2006). Interactions between bacteria and eukaryotes via small molecules. In *Current Opinion in Biotechnology* (pp. 268–273). https://doi.org/10.1016/j.copbio.2006.04.004

El-Demerdash, A.; Tammam, M. A.; Atanasov, A. G.; Hooper, J. N. A.; Al-Mourabit, A. and Kijjoa, A. (2018). Chemistry and biological activities of the marine sponges of the genera mycale (Arenochalina), Biemna and Clathria. In *Marine Drugs*. https://doi.org/10.3390/md16060214

Esposito, R.; Ruocco, N.; Viel, T.; Federico, S.; Zupo, V. and Costantini, M. (2021). Sponges and Their Symbionts as a Source of Valuable Compounds in Cosmeceutical Field. In *Marine Drugs*. https://doi.org/10.3390/md19080444

Goel, C.; Shakir, C.; Tesfaye, A.; Raghavanpillai Sabu, K.; Idhayadhulla, A.; Manilal, A.; Woldemariam, M.; Vijayan, N. and Shah, S. (2022). Antibiofilm Potential of Alpha-Amylase from a Marine Bacterium, Pantoea agglomerans. *The Canadian Journal of Infectious Diseases and Medical Microbiology = Journal Canadien Des Maladies Infectieuses et de La Microbiologie Medicale*, *2022*: 7480382. https://doi.org/10.1155/2022/7480382

Gopinath, S. C. B.; Anbu, P.; Arshad, M. K. M.; Lakshmipriya, T.; Voon, C. H.; Hashim, U. and Chinni, S. V. (2017). Biotechnological Processes in Microbial Amylase Production. *BioMed Research International*, *2017*: 1272193. https://doi.org/10.1155/2017/1272193

Guo, S.; Zhang, Z. and Guo, L. (2022). Antibacterial Molecules from Marine Microorganisms against Aquatic Pathogens: A Concise Review. *Marine Drugs*, *20*(4): 230. https://doi.org/10.3390/md20040230

Gupta, P.; Samant, K. and Sahu, A. (2012). Isolation of Cellulose-degrading Bacteria and Determination of their Cellulolytic Potential. *International Journal of Microbiology*, *2012*: 578925. https://doi.org/10.1155/2012/578925

Gupta, R.; Gigras, P.; Mohapatra, H.; Goswami, V. K. and Chauhan, B. (2003). Microbial α-amylases: a Biotechnological Perspective. *Process Biochemistry*, *38*(11): 1599–1616. https://doi.org/10.1016/S0032-9592(03)00053-0

Izzati, F.; Warsito, M. F.; Bayu, A.; Prasetyoputri, A.; Atikana, A.; Sukmarini, L.; Rahmawati, S. I. and Putra, M. Y. (2021). Chemical diversity and biological activity of secondary metabolites isolated from indonesian marine invertebrates. In *Molecules*. https://doi.org/10.3390/molecules26071898

Jakubiec-Krzesniak, K.; Rajnisz-Mateusiak, A.; Guspiel, A.; Ziemska, J. and Solecka, J. (2018). Secondary metabolites of actinomycetes and their antibacterial, antifungal and antiviral properties. *Polish Journal of Microbiology*, *67*(3): 259–272. https://doi.org/10.21307/pjm-2018-048

Jim, O. (2016). Jim O'Neill. *Nature Reviews. Drug Discovery*, *15*(526). https://doi.org/10.1038/nrd.2016.160

John J, A.; Samuel, M. S.; Govarthanan, M. and Selvarajan, E. (2022). A Comprehensive Review on Strategic Study of Cellulase Producing Marine Actinobacteria for Biofuel Applications. *Environmental Research*, *214*: 114018. https://doi.org/10.1016/j.envres.2022.114018

Kibungu, W. C.; Clarke, A. M.; Fri, J. and Njom, H. A. (2021). Antimicrobial Potential and Phytochemical Screening of Clathria sp. 1 and Tedania (Tedania) stylonychaeta Sponge Crude Extracts Obtained from the South East Coast of South Africa. *BioMed Research International*. https://doi.org/10.1155/2021/6697944

Kim, S.; Lee, J. Y.; Jeong, Y. and Kang, C.-H. (2022). Antioxidant Activity and Probiotic Properties of Lactic Acid Bacteria. *Fermentation*, *8*(1), 29. https://doi.org/10.3390/fermentation8010029

Krishna M. S.; A.; Mohan, S.; Ashitha, K. T.; Chandramouli, M.; Kumaran, A.; Ningaiah, S.; Babu, K. S. and Somappa, S. B. (2022). Marine Based Natural Products: Exploring the Recent Developments in the Identification of Antimicrobial Agents. *Chemistry and Biodiversity*, *19*(10). https://doi.org/10.1002/cbdv.202200513

Larsson, D. G. J. and Flach, C. F. (2022). Antibiotic Resistance in The Environment. In *Nature Reviews Microbiology*. https://doi.org/10.1038/s41579-021-00649-x

León-Palmero, E.; Joglar, V.; Álvarez, P. A.; Martín-Platero, A.; Llamas, I. and Reche, I. (2018). Diversity and Antimicrobial Potential in Sea Anemone and Holothurian Microbiomes. *PLOS ONE*, *13*(5): e0196178. https://doi.org/10.1371/journal.pone.0196178

Li, P.; Lu, H.; Zhang, Y.; Zhang, X.; Liu, L.; Wang, M. and Liu, L. (2023). The Natural Products Discovered in Marine Sponge-associated Microorganisms: Structures, Activities, and Mining Strategy. *Frontiers in Marine Science*, *10*. https://doi.org/10.3389/fmars.2023.1191858

Maharsiwi, W.; Astuti, R. I.; Meriyandini, A. and Wahyudi, A. T. (2020). Screening and Characterization of Sponge-associated Bacteria from Seribu Island, Indonesia Producing Cellulase and Laccase Enzymes. *Biodiversitas Journal of Biological Diversity*, *21*(3). https://doi.org/10.13057/biodiv/d210317

Mehta, A.; Guleria, S.; Sharma, R. and Gupta, R. (2021). The Lipases and Their Applications with Emphasis on Food Industry. In *Microbial Biotechnology in Food and Health* (pp. 143–164). Elsevier. https://doi.org/10.1016/B978-0-12-819813-1.00006-2

Muller, W. G.; Grebenjuk, V.; Le Pennec, G.; Schroder, H.-C.; Brummer, F.; Hentschel, U.; Muller, I. and Breter, H.-J. (2004). Sustainable Production of Bioactive Compounds by Sponges?Cell Culture and Gene Cluster Approach: A Review. *Marine Biotechnology*, *6*(2). https://doi.org/10.1007/s10126-002-0098-6

Nofiani, R.; Weisberg, A. J.; Tsunoda, T.; Panjaitan, R. G. P.; Brilliantoro, R.; Chang, J. H.; Philmus, B. and Mahmud, T. (2020). Antibacterial Potential of Secondary Metabolites from Indonesian Marine Bacterial Symbionts. *International Journal of Microbiology*, *2020*: 1–11. https://doi.org/10.1155/2020/8898631

Patel, N.; Rai, D.; Shivam, Shahane, S. and Mishra, U. (2019). Lipases: Sources, Production, Purification, and Applications. *Recent Patents on Biotechnology*, *13*(1): 45–56. https://doi.org/10.2174/1872208312666181029093333

Phui-Chyng, Y.; Noorfazlin, A.; Woon, J. J.; Cindy, S. J. T.; Vannajan, S. L.; Adzzie Shazleen, A.; Sazaly, A. and Lee, H. Y. (2021). Profiling of Potential Antibacterial Compounds of Lactic Acid. *Molecules*, *26*(1727): 1–14.

Priyanka, P.; Kinsella, G.; Henehan, G. T. and Ryan, B. J. (2019). Isolation, Purification and Characterization of a Novel Solvent Stable Lipase from Pseudomonas Reinekei. *Protein Expression and Purification*, *153*: 121–130. https://doi.org/10.1016/j.pep.2018.08.007

Queiroga, A. C.; Pintado, M. E. and Malcata, F. X. (2012). Search for Novel Proteolytic Enzymes Aimed at Textile and Agro-Industrial Applications: An Overview of Current and Novel Approaches. *Biocatalysis and Biotransformation*.

https://doi.org/10.3109/10242422.2012.650856

Raveendran, S.; Parameswaran, B.; Ummalyma, S. B.; Abraham, A.; Mathew, A. K.; Madhavan, A.; Rebello, S. and Pandey, A. (2018). Applications of Microbial Enzymes in Food Industry. *Food Technology and Biotechnology*, *56*(1): 16–30. https://doi.org/10.17113/ftb.56.01.18.5491

Razzaq, A.; Shamsi, S.; Ali, A.; Ali, Q.; Sajjad, M.; Malik, A. and Ashraf, M. (2019). Microbial Proteases Applications. *Frontiers in Bioengineering and Biotechnology*, *7*. https://doi.org/10.3389/fbioe.2019.00110

Rudi, A.; Yosief, T.; Loya, S.; Hizi, A.; Schleyer, M. and Kashman, Y. (2001). Clathsterol, a Novel Anti-HIV-1 RT Sulfated Sterol from The Sponge Clathria Species. *Journal of Natural Products*. https://doi.org/10.1021/np010121s

Schröder, H. C.; Brümmer, F.; Fattorusso, E.; Aiello, A.; Menna, M.; de Rosa, S.; Batel, R. and Müller, W. E. G. (2003). Sustainable Production of Bioactive Compounds from Sponges: Primmorphs as Bioreactors. *Progress in Molecular and Subcellular Biology*, *37*: 163–197. https://doi.org/10.1007/978-3-642-55519-0_7

Solanki, P.; Putatunda, C.; Kumar, A.; Bhatia, R. and Walia, A. (2021). Microbial Proteases: Ubiquitous Enzymes with Innumerable Uses. *3 Biotech*, *11*(10): 428. https://doi.org/10.1007/s13205-021-02928-z

Souza, P. M. and Magalhães, P. (2010). Application of Microbial α-amylase in Industry - A review. *Brazilian Journal of Microbiology*, *41*(4): 850–861. https://doi.org/10.1590/S1517-83822010000400004

Srinivasan, R.; Kannappan, A.; Shi, C. and Lin, X. (2021). Marine Bacterial Secondary Metabolites: A Treasure House for Structurally Unique and Effective Antimicrobial Compounds. *Marine Drugs*, *19*(10): 530. https://doi.org/10.3390/md19100530

Stincone, P. and Brandelli, A. (2020). Marine Bacteria as Source of Antimicrobial Compounds. *Critical Reviews in Biotechnology*, *40*(3): 306–319. https://doi.org/10.1080/07388551.2019.1710457

Sulistiyani, S.; Ardyati, T. and Winarsih, S. (2016). Antimicrobial and Antioxidant Activity of Endophyte Bacteria Associated with Curcuma longa Rhizome. *The Journal of Experimental Life Sciences*, *6*(1): 45–51. https://doi.org/10.21776/ub.jels.2016.006.01.11

Suriya, J.; Bharathiraja, S.; Krishnan, M.; Manivasagan, P. and Kim, S.-K. (2016). *Marine Microbial Amylases* (pp. 161–177). https://doi.org/10.1016/bs.afnr.2016.07.001

Tavano, O. L.; Berenguer-Murcia, A.; Secundo, F. and Fernandez-Lafuente, R. (2018). Biotechnological Applications of Proteases in Food Technology. *Comprehensive Reviews in Food Science and Food Safety*, *17*(2): 412–436.

https://doi.org/10.1111/1541-4337.12326

The Lancet. (2022). Antimicrobial resistance: time to repurpose the Global Fund. *The Lancet*, *399*(10322), 335. https://doi.org/10.1016/S0140-6736(22)00091-5

Varijakzhan, D.; Loh, J.-Y.; Yap, W.-S.; Yusoff, K.; Seboussi, R.; Lim, S.-H. E.; Lai, K.-S. and Chong, C.-M. (2021a). Bioactive Compounds from Marine Sponges: Fundamentals and Applications. *Marine Drugs*, *19*(5). https://doi.org/10.3390/md19050246

Varijakzhan, D.; Loh, J.-Y.; Yap, W.-S.; Yusoff, K.; Seboussi, R.; Lim, S.-H. E.; Lai, K.-S. and Chong, C.-M. (2021b). Bioactive Compounds from Marine Sponges: Fundamentals and Applications. *Marine Drugs*, *19*(5): 246. https://doi.org/10.3390/md19050246

Viswanathan, K. and Rebecca, L. J. (2019). Screening of Amylase and Cellulase Enzymes from Marine Actinomycetes. *Research Journal of Pharmacy and Technology*, *12*(8): 3787. https://doi.org/10.5958/0974-360X.2019.00648.6

World Health Organization. (2021). *Antimicrobial Resistance*. World Health Organization. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance

Xing, J.; Wang, G.; Zhang, Q.; Liu, X.; Gu, Z.; Zhang, H.; Chen, Y. Q. and Chen, W. (2015). Determining Antioxidant Activities of Lactobacilli Cell-Free Supernatants by Cellular Antioxidant Assay: A Comparison with Traditional Methods. *PLOS ONE*, *10*(3): e0119058. https://doi.org/10.1371/journal.pone. 0119058

Zhang, G. and Dong, Y. (2022). Design and Application of An Efficient Cellulose-degrading Microbial Consortium and Carboxymethyl Cellulase Production Optimization. *Frontiers in Microbiology*, *13*. https://doi.org/10.3389/fmicb. 2022. 957444