BIODIVERSITAS
Volume 24, Number 1, January 2023
Pages: 20-25

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d240103

Enzyme-producing symbiotic bacteria in gastropods and bivalves molluscs: Candidates for bioindustry materials

WILIS ARI SETYATI♥, DELIANIS PRINGGENIES, NIRWANI SOENARDJO, RINI PRAMESTI
Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Diponegoro. Jl. Prof. H. Soedarto, S.H, Semarang 50275, Central Java, Indonesia. Tel./fax.: +62-24-7474698, ♥email: wilisarisetyati@yahoo.co.id

Manuscript received: 3 November 2022. Revision accepted: 24 December 2022.

Abstract. *Setyati WA, Pringgenies D, Soenardjo N, Pramesti R. 2023. Enzyme-producing symbiotic bacteria in gastropods and bivalves molluscs: Candidates for bioindustry materials. Biodiversitas 24: 20-25.* Biotechnology plays a vital role in modern civilization because almost all aspects of human life have benefited from the development of biotechnological applications, especially in the bioindustry. Therefore, the present study aims to obtain bacterial isolates from molluscs and identify those with the potential to produce protease, amylase, and cellulase enzymes using bimolecular methods and bacterial biochemical analysis. The samples were collected in April 2022 from the coastal waters of Krakal, Special Region of Yogyakarta, Indonesia. The symbiotic bacteria were isolated, and screened for different enzymatic activity. The molecular method (16S rRNA gene sequence) was used to identify bacterial isolates with the highest potential for enzymatic activity. Biochemical analysis of isolates that are potential candidates for industrial materials was carried out using the thin-Layer Chromatography (TLC) method to detect the compounds produced by bacteria. The results showed that the bacterial isolates GS 1-4, GS 2-1, and GS 2-12 had similarity with *Alcaligenes faecalis* (99.83%), *Alcaligenes faecalis* (99.74%) and *Alcaligenes aquatilis* (98.51%) respectively. The three isolates showed the potent enzymatic activity and contained alcohol, amines, aldehyde, ketone and conjugated aromatics, as well as amino acids for potential candidates of bioindustry materials.

Keywords: Bacteria, bioindustry, enzymatic, molluscs, PCR, TLC

INTRODUCTION

The bioindustry is an inseparable part of modern civilization due to the continuous use of bioindustry techniques in the production of processed products such as wine, bread, and other foodstuffs. The industrial sector is important in agriculture for the realization of a sustainable the bioindustry farming system as the main vision of agricultural development for 2015-2045. Along with the development of the bioindustry, enzymes become one of the important raw materials. Previous research showed that more than 80% of the global enzyme market consists of products from the application of catalysts (Adrio and Demain 2014; Singh et al. 2016). Although more than 50% of these enzymes are obtained from genetically modified organisms, their uses are still concentrated in the food industry (Singh et al. 2018).

Molluscs are one of the most common types of marine organisms found in intertidal areas (Ariyanto et al. 2020) and have antimicrobial potency (Pringgenies et al. 2021a). They have high potential as a source of bioactive agents commonly used in the bioindustry but, overexploitation has threatened their existence in the wild. Excessive sourcing from natural habitats can also cause a significant reduction in the number of these organisms and an imbalance in the ecosystem. This leads to the investigation of the potential for bacterial symbionts of marine organisms. Subsequently, it was discovered that, symbiotic bacteria have great potential in producing the same active compounds (Kristiana et al. 2020). Therefore, symbiotic bacteria could be used as a substitute for various kinds of organisms as a source of exploitable raw materials. By applying bacterial symbionts as a source, the bioindustry could be meet the need for enzyme raw materials in a sustainable manner, which aids the survival of their host species (Zhukova et al. 2022).

More than 80% of the global enzymes market is derived from the applications of catalysts in the bioindustry. It was found that, the ocean holds enormous potential because only about 30% of marine resources are currently used in biotechnology. Meanwhile, the direct exploitation of organisms to produce enzymes can threaten the survival of certain endangered species. Garcia and Gerardo (2014) have emphasized the use of bacterial symbiont organisms as a substitute for the original microorganism in sourcing materials. This is because the organisms contain the same compounds as the host and can be replenished by culture in a controlled environment within a relatively short period.

The use of symbiotic bacteria in replacing the role of native organisms in synthesizing enzymes have distinct advantages. These include the prevention of overexploitation of the host organism, and meeting the demands of enzymes, especially protease and amylase in the market. This is because microorganisms have a high level of activity, coupled with a fast growth rate, and can produce the enzyme amylase in higher quantities (Gopinath et al. 2013). Microorganisms are also used as a source of enzymes (Raveendran et al. 2018), because of a shorter production cycle, ease of cultivation and modification, relatively fast growth rate, low production costs, and their

availability does not depend on changing seasons (Narihiro and Kamagata 2013; Lobo et al. 2019, Bonnet et al. 2020). Other results showed that fast-growing engineered microbes (Pei and Schmidt 2018), microorganisms and climate change (Cavicchioli et al. 2019), symbiotic microbiome (Yang et al. 2022), large-scale production of enzymes (Fasim et al. 2021), characteristics of lactic acid bacteria (LAB) and the expanding applications in the food industry (Wang et al. 2021) and molluscan compounds provide drug leads (Bonnet et al. 2020). Therefore, the present study aims to determine the potential of gastropods and bivalve molluscs symbiotic bacteria that produce protease, amylase, and cellulase enzymes in the coastal waters of Krakal, Yogyakarta, Indonesia, using molecular identification.

MATERIALS AND METHODS

Study area

Samples were collected in April 2022, including two gastropod species, namely *Conus ebraeus* L.1758 and *Morula aspera*, as well as one bivalve species, *Hiatula chinensis* collected from Krakal beach, Yogyakarta. Indonesia.

Isolation and characterization, of symbiotic bacteria

The media used to isolate bacteria was Zobell marine broth 2216E with the addition of 3% agar. The media was homogenized using a magnetic stirrer at a temperature of ± 80^0C and a magnet, followed by sterilization using an autoclave for 15 minutes at 121°C. Subsequently, 10 g of the sample was taken and put into 90 mL of sterile seawater, which was diluted to a factor of 10^{-1}, 10^{-2}, and 10^{-3}. Hundred microliters from each dilution was taken and introduced onto Zobell marine 2216 E agar media. The mixture was flattened using a spreader and incubated for 48 h at room temperature ranging from 20-25°C. Purification of bacterial isolates was carried out using the streak method by Setyati and Subagiyo (2012). Bacterial colonies were categorized and selected based on morphology and purified by streak plates method to obtain pure cultures. Subsequently, bacterial characteristics were determined by direct observation (macroscopic), namely colony shape, surface type, edge, and color, which were further classified.

Screening of bacteria for enzyme activity

The enzymatic activity of pure cultures was determined by testing the production activity of the enzymes protease, amylase, and cellulase using the procedures by Setyati et al. (2016) with slight modifications. Screening for proteolytic activity was carried out by culturing the test bacteria on Zobell marine agar media enriched with 1% skim milk, and the plates were incubated for 48 h. The bacteria were spotted onto Petri plates using the doting technique and proteolytic activity was examined by transparent zone formation. The activity of the amylase enzyme was carried out by culturing the test bacteria using the doting technique on Zobell marine agar media, which was enriched with 1% soluble starch. Bacterial growth was observed after the incubation process for 48 h and the media was dripped with iodine to determine the amylolytic activity based on the clear zone formed. Cellulase activity was tested by culturing the bacteria on Zobell marine agar media enriched with 1% carboxymethylcellulose (CMC). Subsequently, bacteria were cultured using the doting technique and the media were incubated within 48 h. After incubation time, iodine was dripped onto the media to determine cellulosic activity.

Characterization of compounds produced by bacteria

Thin-layer chromatography (TLC) method was employed to detect the compounds produced by bacteria. The TLC plate was inserted into a glass chamber containing n-hexane in liquid and saturated vapor form. The N-hexane will move along the plate, which was removed from the chamber when the n-hexane reaches the upper limit of the plate end. Observation of the sample elution was carried out using UV light at wavelengths of 254 nm and 330 nm. The appearing spots were marked with a pencil and the elution results were further observed by dipping the TLC plate. KMnO4 stain solution were dried to emphasize the spots. The experiment was repeated for n-hexane that was added with ethyl acetate, dichloromethane (DCM), chloroform, or acetone in various volume ratios.

Microscopic characterization of bacteria

The three bacteria with the highest enzymatic activity were selected to pass through the Gram staining process that was carried out using stain reagents consisting of A (Violet crystal), B (Lugol), and C (Safranin). The bacterial sample were first placed on an object of glass and dried over a fire. Subsequently, reagent A was dripped on the slide for 30 seconds and the slide was rinsed with running water and drain. The reagent B was introduced to the preparation and left for 30 seconds, which was rinsed and dried. The preparation was rinsed with 70% alcohol until the color was not dissolved, cleansed again with running water, and allowed to dry. Reagent C was dripped onto the surface of the preparation and allowed to stand for 1 min, rinsed, and dried. The staining results were observed under a microscope with a magnification of 100X 1.25 and assisted with immersion oil.

Molecular identification

Molecular identification was completed through the initial extraction of the DNA from bacteria. The extracted DNA template was put into the polymerase chain reaction (PCR) process using the standard method. This was followed by electrophoresis observation of the agarose gel and the results were viewed using a UV transilluminator. The results of the PCR product were sent to PT, Genetika Science Indonesia, in Jakarta for sequencing. The finished sequence data were edited using MegaX software and compared with the existing sequence data in GenBank using BLAST (Basic Local Alignment Search Tool) in the NCBI database (http://www.ncbi.nlm.nih.gov). The similarity of the 16S rRNA gene sequences results with the NCBI database sample is determined from the similitude of

the total score, query cover, e-value, and percentage of the bacteria.

RESULTS AND DISCUSSION

Isolation and characterization of symbiotic bacteria

The symbiotic bacteria isolated from the samples had different morphologies. Based on observations, there were 55 bacterial colonies from the collected samples with 17 GS 1, 13 GS 2, 10 BV 1, and 15 BV 2 symbionts. The enzymatic activity such as protease, amylase, and cellulase of the isolated symbiotic bacteria observed showed varying results among the four samples observed. It was also discovered that 12 bacteria were able to synthesize protease enzymes, while 10 bacteria synthesized amylase enzymes. However, there were no bacteria capable of synthesizing cellulase enzymes (Tables 1).

The results of the enzymatic test showed that there are 3 selected isolates to be namely isolates GS 1-4; GS 2-1and GS 2-12 were evaluated for further study. Gram staining was performed on three selected bacterial isolates that had the highest enzymatic activity. All three selected isolate were Gram negative bacteria.

Molecular identification of symbiotic bacteria

The search results for the homology of the 16S rRNA gene sequence of symbiotic bacteria isolates with BLASTn showed that the GS 1-4 match (99.83%) with *Alcaligenes faecalis*, isolates GS 2-1 and GS 2-12 had a match of 99.74% and 98.51% with *A. faecalis*, respectively (Table 2).

Characterization of compounds produced by bacteria

Based on the results of the TLC (Table 3), bacterial isolate GS 1-4 contains alcohols, amines, aldehydes, ketones and amino acids as well as aromatics conjugated compounds. Bacterial isolate GS 2-1 contains alcohols, amines, aldehydes, ketones, and aromatics conjugated compounds. Meanwhile, bacterial isolate GS 2-12 contains only aromatics conjugated compounds.

The abundance of molluscs in the inter-tidal area is influenced by various factors, which affected other species specific to each coast. Characteristics and changes in their environment cause molluscs to adapt for their survival. This condition allows marine biota such as molluscs to produce secondary metabolites that are quite diverse as a means of survival.

Meanwhile, the restriction on the movement of animals has the potential for more promising bioactive compounds when compared to those that move freely (Santosa et al. 2020; Pringgenies et al. 2021b). Since enzymes are widely used components in the bioindustry today, there is a real concern that using natural sources can lead to the overexploitation of organisms. Symbiotic bacteria are associated with both the outside (epiphytic) and inside (endophytic) of an organism, with the ability to produce compounds that are similar or even the same as their hosts.

The use of symbiotic bacteria to replace organisms as a source of various compounds has been put forward as a solution to prevent and mitigate the impact of overexploitation of natural resources. Krakal Beach, Gunung Kidul, Yogyakarta, Indonesia has a high diversity of mollusc species, with an elevated adaptation cycle and a fairly extreme environment on the southern coast of Java. The molluscs in this area are estimated to have high adaptability and produce compounds, and good enzymes, which makes them important for research.

Table 1. Enzymatic activity of symbiotic bacteria

Bacteria	Enzymatic activity	
	Protease	Amylase
GS 1-2	-	+
GS 1-4	+	+
GS 1-5	+	+
GS 1-9	+	+
GS 1-10	-	+
GS 1-11	-	+
GS 2-1	+	+
GS 2-4	+	+
GS 2-9	+	-
GS 2-10	+	-
GS 2-12	+	+
GS 2-13	+	+
BV 1-2	+	-
BV 1-4	+	-
BV 2-3	+	-

Table 2. BLAST homology of symbiont bacteria of gastropods and bivalves molluscs

Isolate	Relative match	Homology (in %)	Access number
GS 1-4	*Alcaligenes faecalis*	99.83%	MT579857.1
GS 2-1	*Alcaligenes faecalis*	99.74%	MT579857.1
GS 2-12	*Alcaligenes aquatilis*	98.51%	MT572474.1

Table 3. Results of thin-layer chromatography (TLC) on the selected bacteria

Bacterial isolate	Staining	Detection
GS 1-4	Vainillin	Alcohol, amines, aldehyde, ketone
	Ninhidrin	Amino acid
	UV	Aromatics conjugated
GS 2-1	Vainillin	Alcohol, amines, aldehyde, ketone
	Ninhidrin	-
	UV	Aromatics conjugated
GS 2-12	Vainillin	-
	Ninhidrin	-
	UV	Aromatics conjugated

The results obtained after the process of characterizing the symbiotic bacteria in the entire sample showed diversity. Isolation data and characterization of GS 1 symbiotic bacteria revealed that the characteristics of the isolate colonies were irregular and circular forms, with entire edges and raised surfaces. The dominant color from the overall isolation is yellow, with a spectrum from bright yellow to neon. In the GS 2 sample, the symbionts were dominated by bacterial colonies with a circular form, entire edges, and raised surfaces. The dominant isolate colonies showed yellow and white coloration.

Sample BV 1 produced symbiotic bacteria with a circular shape, entire edge, and a raised surface, where yellow is the dominant color which varies in shades. Meanwhile, sample BV 2 had colonies with punctiform morphology, entire edges, and raised surfaces, which are dominated by yellow and white. Differences in the characteristics and colors of bacterial colonies are caused by several internal and external factors. The difference in growth in the test media occurred due to the nutrients contained. According to Gu et al. (2021), the differences in the growth of bacterial colonies were caused by variations in the density of bacterial cells and the availability of nutrients in the culture media. Bacterial characterization was carried out after isolation and before purification to enable better observation of the shape of the colonies' growth. Subsequently, bacterial growth tends to follow the streak of the inoculation line and spread evenly based on the availability of nutrient content (Singh et al. 2016).

Enzymatic activity was shown by several bacterial colonies, although there were colonies that did not show any activity. The activity of the protease enzyme was shown by 9 gastropods and 3 bivalve symbionts, where the highest protease activity was found by the *A. faecalis* GS 2-1. This conclusion was obtained from the observation of the *A. faecalis* GS 2-1 inhibitory zone, which was the clearest and largest among other symbiotic bacteria isolates. Other isolates showed inhibitory zones that indicated the rest of the abilities. However, the size of the inhibitory zone can not be calculated quantitatively because of the difference in growth rates between one bacterium and another. The appearance of an inhibitory zone around the bacteria indicated the ability to hydrolyze the skim milk content in the growth media. Moreover, the ability of isolates to break down protein content in skim milk is directly proportional to the transparency of the inhibitory zone around bacterial growth. These results showed that not all gastropod and bivalve symbiotic bacteria have protease enzyme activity. It was also observed that bacteria isolated from gastropods have a higher potential to synthesize protease enzymes as indicated by the greater number of isolates that showed positive results in the activity screening.

The ability of bacteria to secrete protease enzymes is influenced by several factors. Bacterial isolates without an inhibitory zone were an indication that these species cannot secrete protease enzymes due to several factors that cannot be clarified through testing only. Enzymes are primary metabolites formed in the logarithmic growth phase (Tamano 2014). In this phase, cells will grow rapidly for enzyme production to increase significantly. Therefore, further research on the factors influencing the activity of protease enzyme production in symbiotic bacteria can be a valuable contribution to science and technology. In testing the production of protease enzymes, it is necessary to analyze the protein content and determine the specific activity of the related enzyme per milligram of protein (Fachrial et al. 2021).

In observing the amylase enzymatic activity, it was discovered that the symbiotic bacteria capable of secreting this enzyme were 10 bacterial colonies from gastropod samples. The highest amylase enzyme activity was indicated by *A. faecalis* GS 1-4 as shown macroscopically from the diameter of the inhibitory zone and the level of transparency on the media. The transparent zone is formed due to the degradation of starch in the media, under the influence of the activity of the amylase enzyme. The clear zone formed will be visible on the agar around the bacterial colonies when an iodine solution is introduced to the surface.

Testing of amylase activity was carried out by growing test isolates on media that had been added with starch. Since pure microorganism isolate can be cultured using media containing starch, iodine was introduced to the surface of the culture media impregnated with bacterial isolates. When there is a transparent zone, it indicates that the bacteria produce amylase enzyme because in the clear area the starch has been hydrolyzed, while the blue-black area shows the opposite. In cellulosic activity testing, no bacterial isolates produce cellulase enzymes. The cellulase activity was tested by culturing the test bacteria on Zobell marine agar media enriched with 1% CMC. Observations were made by dripping iodine onto the surface of the culture medium and the presence of cellulosic activity is indicated by the formation of a transparent zone. Based on observations, none of the bacteria were able to secrete cellulase enzymes.

Several types of enzymes commonly used in the bioindustry are protease enzymes, catalase, AMP deaminase, galactosidase, glucanase, invertase, maltase, zymase, dextranase, glucoamylase, pectinase, lactase, amylase, and cellulase (Raveendran et al. 2018). Meanwhile, protease, amylase, and cellulase enzymes are enzymes that are quite often used in processed products and are also often found in the public (Nigam 2013). The three isolates of symbiotic bacteria with the highest enzymatic activity were selected for molecular identification followed by BLAST homology matching to determine the identified species. The most viable isolates were *A. faecalis* GS 2-1, *A. faecalis* GS 1-4, and *A. aquatilis* GS 2-12 for the best proteolytic, amylolytic isolate, as well as proteolytic and amylolytic, respectively. Factors considered in selecting isolates were based on the size and level of the transparency zone formed, as well as the stability of the determining enzymatic activity.

Amylase enzymes are widely used in the manufacture of bread in various types of industries and in the fermentation process of several types of foodstuffs. Amylase is estimated to represent 30% of enzyme production worldwide. Meanwhile, cellulase enzymes are

used in the bioindustry, especially in food processing such as coffee and pharmaceuticals (Ejaz et al. 2021). It was discovered that enzymes in the digestive tract, including cellulase enzymes, can produce coffee with a distinctive aroma and taste. Enzymes are compounds produced by microorganisms, plants and animals. Natural materials consisting of living things, such as flora and fauna can also produce enzymes to support their growth, whereas some species of organisms produce specific enzymes due to several factors. Animals have great potential in producing enzymes, with various species contributing to the need for enzymes in the bioindustry field. Although the fauna is known to be sourced from land or sea, water and marine areas have high diversity and potential. It is also reported that the sea is the habitat of a wide variety of flora and fauna, however, only about 30% of the sea has been explored for this purpose.

The results of the Gram staining of the three bacteria showed a red color in the cell samples, therefore, they are Gram-negative. Gram-stained bacteria can be observed using oil immersion and a microscope with a magnification of 40 x 100. Oil immersion is used to clarify the object under the microscope. The oil will increase the light that enters the objective lens after passing through the object. The difference in the results of Gram staining is caused by variations in the composition of the bacterial cell wall. The results showed that the walls of Gram-positive bacteria are mostly (90%) composed of peptidoglycan, while the rest is teichoic acid. The Gram-positive bacteria produce purple stains due to the presence of complex bonds with crystal violet, while the cell walls of Gram-negative bacteria only contain 5-20% peptidoglycan and the rest contain polysaccharides. At the time of Gram staining, the administration of an alcohol solution can dissolve the lipids in the cell's outer membrane for the purple color of the crystal violet to fade and the cell will become colorless. When stained in red from safranin, the cells will absorb the color, thereby Gram-negative bacteria turn red.

Molecular identification was carried out to determine the identified species of symbiotic bacteria isolates. Based on the results, isolates GS 2-1 and GS 1-4 were similar, namely, *A. faecalis*, while GS 2-12 was *A. aquatilis*. These two species have a very high similarity because they are both members of the same genus. *Alcaligenes aquatilis* is Gram-negative, aerobic, and motile bacteria but the former is known as a pathogenic bacterium. They can cause infection on the skin surface and soft tissues, this is confirmed by Tena et al. (2015), who found that *A. faecalis* has a high potential as a bacterium that causes infection in patients with vascular disease or after surgery, especially in the skin and soft tissues. According to Hasan et al. (2019), *A. faecalis* is non-pigmented or sometimes yellow and commonly found in the environment, both in soil and water. This species is not usually considered a pathogen, but in some cases of opportunistic infections, and is pathogenic to fish. *A. aquatilis* is commonly found in areas near waters or oceans. These bacteria grow in an environment with a temperature range of 4 to 35°C with an optimal value of 18 to 24°C. *A. aquatilis* can reduce nitrate and nitrite under anaerobic conditions, which affect the growth rate of bacterial isolates, while *A. faecalis* enhanced denitrification rate with electrodes as the electron donor (Wang et al. 2015).

Several factors that caused the presence of these bacteria in the molluscs tested at Krakal Beach, Yogyakarta was due to intensive human activities in the coastal area. The two bacteria have been carried from the inland and reached the coastal area, which formed a symbiosis with the molluscs in the area. Based on the results of the TLC test on the 3 isolate samples, it was discovered that *A. faecalis* GS 2-1 contains alcohols, amines, aldehydes, ketones, and Aromatics Conjugated compounds. *A. aquatilis* GS 2-12 contains only Aromatics Conjugated compounds, while *A. faecalis* GS 1-4 contains alcohols, amines, aldehydes, ketones, and amino acids as well as aromatics conjugated compounds. Generally, each isolate contains aromatics conjugated, two isolates contain alcohols, amines, aldehydes, ketones, and only one isolate has amino acids.

Alcohols, amines, aldehydes, ketones, and amino acids as well as aromatic compounds are compounds contained in enzymes that help the formation of enzymes. Among the constituents of this acid are vanillylamine, thyroxine, and tryptophan, which also make up enzymes, while medium alcohol compounds contain serine and threonine. Amines are groups that are present in enzymes, which are their constituent compounds. This can be found in the enzymatic reactions developed for the synthesis of alcohols from simple aldehydes and amines, by combining the reactions of hydroxymethylation and reduction amination. This methodology was applied to rapidly access a key building block of various pharmaceutically important tetrahydro-quinoline alkaloids (Zhan et al. 2022).

In conclusion, the bacterial isolate GS 2-12 had 98.51% similarities with the type of *A. aquatilis*. Meanwhile, isolates GS 2-1 and GS 1-4 had 99.83% and 97.74% match with *A. faecalis*, respectively. The three isolates showed the required enzymatic activity and contained alcohol, amines, aldehyde, ketone and conjugated aromatics, as well as amino acids for potential candidates of bioindustry materials.

ACKNOWLEDGMENTS

The authors are grateful to the Faculty of Fisheries and Marine Sciences, Diponegoro University, Semarang, Indonesia for providing instruments and research funding (No: 64/UN7.5.10.2/HK/2022).

REFERENCES

Adrio JL, Demain AL. 2014. Microbial enzymes: tools for biotechnological processes. Biomolecules 4 (1): 117-139. DOI: 10.3390/biom4010117.

Ariyanto D, Bengen DG, Prartono T, Wardiatno Y. 2020. Distribution and abundance of *Cerithideopsilla djadjariensis* (Martin 1899) (Potamididae) on *Avicennia marina* in Rembang, Central Java, Indonesia. Egypt J Aquat Biol Fish 24 (3): 323-332. DOI: 10.21608/EJABF.2020.95329.

Bonnet M, Lagier JC, Raoult D, Khelaifia S. 2020. Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. New Microbes New Infect 34: 100622. DOI: 10.1016/j.nmni.2019.100622.

Cavicchioli R, Ripple WJ, Timmis KN, Azam F, Bakken LR, Baylis M, Behrenfeld MJ, Boetius A, Boyd PW, Classen AT, Crowther TW. 2019. Scientists' warning to humanity: microorganisms and climate change. Nat Rev Microbiol 17 (9): 569-586. DOI: 10.1038/s41579-019-0222-5.

Ejaz U, Sohail M, Ghanemi A. 2021. Cellulases: From bioactivity to a variety of industrial applications. Biomimetics 6 (3): 44. DOI: 10.3390/biomimetics6030044.

Fachrial E, Krisdianilo V, Harmileni, Lister INE, Nugroho TT, Saryono. 2021. Isolation, characterization, activity test and molecular identification of thermophilic bacteria producing proteases from Dolok Tinggi Raja Natural Hot Springs, North Sumatra, Indonesia. Biodiversitas 22: 1725-1732 . DOI: 10.13057/biodiv/d220416.

Fasim A, More VS, More SS. 2021. Large-scale production of enzymes for biotechnology uses. Curr Opin Biotechnol 69: 68-76. DOI: 10.1016/j.copbio.2020.12.002.

Garcia JR, Gerardo NM. 2014. The symbiont side of symbiosis: Do microbes really benefit? Front Microbiol 5: 510. DOI: 10.3389/fmicb.2014.00510.

Gopinath SCB, Anbu P, Lakshmipriya T, Hilda A. 2013. Strategies to characterize fungal lipases for applications in medicine and dairy industry. BioMed Res Intl 2013: 31-34. DOI: 10.1155/2013/154549.

Gu Y, Yan D, Wu M, Li M, Li P, Wang J, Chang Y, Yang F, Di S, Ni S, Yang M, Liu J. 2021. Influence of the densities and nutritional components of bacterial colonies on the culture-enriched gut bacterial community structure. AMB Expr 11: 78. DOI: 10.1186/s13568-021-01240-6.

Hasan MJ, Nizhu LN, Rabbani R. 2019. Bloodstream infection with pandrug-resistant *Alcaligenes faecalis* treated with double-dose of tigecycline. IDCases 18: e00600. DOI: 10.1016/j.idcr.2019.e00600.

Kristiana R, Bedoux G, Pals G, Mudianta IW, Taupin L, Marty C, Asagabaldan MA, Ayuningrum D, Trianto A, Bourgougnon N, Radjasa OK. 2020. Bioactivity of compounds secreted by symbiont bacteria of Nudibranchs from Indonesia. PeerJ 2020 (1): 1-23. DOI: 10.7717/peerj.8093.

Lobo CB, Juárez TMS, Viruel E, Ferrero MA, Lucca ME. 2019. Development of low-cost formulations of plant growth-promoting bacteria to be used as inoculants in beneficial agricultural technologies. Microbiol Res 219: 12-25. DOI: 10.1016/j.micres.2018.10.012.

Narihiro T, Kamagata Y. 2013. Cultivating yet-to-be cultivated microbes: The challenge continues. Microbes Environ 28 (2): 163-165. DOI: 10.1264/jsme2.ME2802rh.

Nigam PS. 2013. Microbial enzymes with special characteristics for biotechnological applications. Biomolecules 3 (3): 597-611. DOI: 10.3390/biom3030597.

Pei L, Schmidt M. 2018. Fast-growing engineered microbes: New concerns for gain-of-function research? Front Genet 9: 207. DOI: 10.3389/fgene.2018.00207.

Pringgenies D, Setyati WA, Djunaedi A, Pramesti R, Rudiyanti S, Ariyanto D. 2021a. Exploration of antimicrobial potency of mangrove symbiont against multi-drug resistant bacteria. Sci J Fish Mar 13 (2): 222-232. DOI: 10.20473/jipk.v13i2.26199.

Pringgenies D, Santosa GW, Yudiati E, Djunaedi A, Ariyanto D. 2021b. The impact of sea cucumber symbiont bacteria *Bacillus aquimaris* and *Virgibacillus chiguensis* on meat quality of salem fish (*Scomber japonicus*). Egypt J Aquatic Biol Fish 25 (2): 237-251. DOI: 10.21608/ejabf.2021.162343.

Raveendran S, Parameswaran B, Ummalyma SB, Abraham A, Mathew AK, Madhavan A, Rebello S, Pandey A. 2018. Applications of microbial enzymes in food industry. Food Technol Biotechnol 56 (1): 16-30. DOI: 10.17113/ftb.56.01.18.5491.

Santosa W, Djunaedi A, Susanto A, Pringgenies D, Ariyanto D. 2020. Characteristics of bioactive compounds of *Holothuria atra* (Jaeger, 1833) associated bacteria. AACL Bioflux 13 (4): 2161-2169.

Setyati WA, Habibi AS, Subagiyo S, Ridlo A, Soenardjo N, Pramesti R. 2016. Screening and selection of sponge symbiont bacteria producing extracellular enzymes as bioremediation agents for organic matter and vibriosis biocontrol in shrimp farming. J Kelaut Trop 19 (1): 11-20. DOI: 10.14710/jkt.v19i1.595.

Setyati WA, Subagiyo. 2012. Isolation and selection of extracellular enzyme producing bacteria originating from mangrove sedimen. Indones J Mar Sci 17 (3): 164-169.

Singh R, Singh A, Sachan S. 2018. Enzymes used in the food industry: Friends or foes? In: Enzymes in Food Biotechnology: Production, Applications, and Future Prospects. Academic Press. DOI: 10.1016/B978-0-12-813280-7.00048-7.

Singh V, Haque S, Singh H, Verma J, Vibha K, Singh R, Jawed A, Tripathi CKM. 2016. Isolation, screening, and identification of novel isolates of actinomycetes from India for antimicrobial applications. Front Microbiol 7: 1921. DOI: 10.3389/fmicb.2016.01921.

Tamano K. 2014. Enhancing microbial metabolite and enzyme production: Current strategies and challenges. Front Microbiol 5: 718. DOI: 10.3389/fmicb.2014.00718.

Tena D, Fernández C, Lago MR. 2015. *Alcaligenes faecalis*: an unusual cause of skin and soft tissue infection. Japan J Infect Dis 68 (2): 128-130. DOI: 10.7883/yoken.jjid.2014.164.

Trappen S. Van Tan T, Samyn E, Vandamme P. 2005. *Alcaligenes aquatili*s sp. nov., a novel bacterium from sediments of the Weser Estuary, Germany, and a salt marsh on Shem Creek in Charleston. Intl J Syst Evol Microbiol 55: 2571-2575. DOI: 10.1099/ijs.0.63849-0.

Wang X, Yu P, Zeng C, Ding H, Li Y, Wang C, Lu A. 2015. Enhanced *Alcaligenes faecalis* denitrification rate with electrodes as the electron donor. Appl Environ Microbiol 81 (16): 5387-5394. DOI: 10.1128/AEM.00683-15.

Wang Y, Wu J, Lv M, Shao Z, Hungwe M, Wang J, Bai X, Xie J, Wang Y, Geng W. 2021. Metabolism characteristics of lactic acid bacteria and the expanding applications in food industry. Front Bioeng Biotechnol 9: 1-19. DOI: 10.3389/fbioe.2021.612285.

Yang MJ, Song H, Feng J, Yu ZL, Shi P, Liang J, Hu Z, Zhou C, Wang XL, Zhang T. 2022. Symbiotic microbiome and metabolism profiles reveal the effects of induction by oysters on the metamorphosis of the carnivorous gastropod *Rapana venosa*. Comput Struct Biotechnol J 20: 1-14. DOI: 10.1016/j.csbj.2021.11.041.

Zhan Z, Xu Z, Yu S, Feng J, Liu F, Yao P, Wu Q, Zhu D. 2022. Stereocomplementary synthesis of a key intermediate for tofacitinib via enzymatic dynamic kinetic resolution-reductive amination. Adv Synth Catal 364 (14): 9-11. DOI: 10.1002/adsc.202200361.

Zhukova NV, Eliseikina MG, Balakirev ES, Ayala FJ. 2022. Multiple bacterial partners in symbiosis with the nudibranch mollusk *Rostanga alisae*. Sci Rep 12 (1): 1-15. DOI: 10.1038/s41598-021-03973-7.