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Production of Some Extracellular Hydrolases by Halophilic Bacteria Isolated from Mangrove Soil in Sumberkima Village, Bali, Indonesia

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ABSTRACT. Hydrolases are enzymes with broad industrial applications across the food, pharmaceutical, textile, detergent, and paper sectors. For industrial applications, microbial enzymes are preferred over plant and animal alternatives due to their robust stability. Halophilic bacteria, which thrive in high-salinity environments, represent a promising yet underutilized source of novel hydrolases. This study aimed to isolate and screen halophilic bacteria from mangrove soil in Sumberkima Village, Buleleng Regency, Bali Province, Indonesia, for their ability to produce some extracellular hydrolases. Soil samples were cultivated on Luria-Bertani (LB) media supplemented with 5% and 10% (w/v) NaCl. Putative colonies were isolated and screened for amylase, cellulase, and lipase production using starch, carboxymethyl cellulose, and rhodamine-olive oil agar, respectively. Enzymatic activities were quantified via spectrophotometry, and the most proficient isolates were identified through 16S rRNA gene phylogenetic analysis. The screening yielded several halophilic bacteria with significant extracellular hydrolase activity. Among the identified isolates, the highest enzymatic activities were observed in *Vibrio xiamenensis* SKMG1(4) for amylase (0.44 U/mL), *Vibrio hepatarius* SKMG1(3) for cellulase (0.36 U/mL), and *Salinivibrio kushneri* SKMG2(5) for lipase (21.6 U/mL). Phylogenetic analysis confirmed the close taxonomic relationship of these isolates to their respective type strains. This study represents the first report of extracellular hydrolase production from these three halophilic bacterial species, highlighting their potential for specialized industrial applications.

Keywords: extracellular hydrolase, halophilic bacteria, mangrove soil

INTRODUCTION

Hydrolases are a class of hydrolytic enzymes extensively utilized across various industrial sectors, including bakery, biofuel, brewing, pharmaceutical, textile, detergent, and paper manufacturing (Yehia et al., 2024). Diverse hydrolases drive these applications. Proteases improve the nutritional value, flavor, solubility, and digestibility of food proteins. Lipases synthesize intermediates for producing active pharmaceutical ingredients. Amylases convert starch to glucose and enhance the taste, crust color, and toasting qualities of bread. Cellulases decompose lignocellulosic material for bioethanol production. Xylanases remove lignin to improve bleaching and fiber properties (Chapman et al., 2018; Raveendran et al., 2018).

Enzymes derived from microorganisms typically exhibit superior stability compared to those sourced from plants or animals, making them highly valuable catalysts across a range of physicochemical conditions (Raveendran et al., 2018; Thapa et al., 2019). Furthermore, microbial enzyme production offers distinct advantages in scalability; it can be achieved efficiently through fermentation techniques that require less time, space, and cost. This process ensures high consistency, facilitating easy modification and optimization for large-scale industrial use (Raveendran et al., 2018). Consequently, the discovery of microbial strains capable of producing novel, superior enzymes remains a central objective for developing industrial enzyme production.

Hydrolase-producing microorganisms can be isolated from various habitats, one of which is mangrove ecosystem. Mangroves represent one of the world's most productive and complex ecosystems. They are characterized by intensive organic matter recycling and nutrient exchange, alongside significant spatial and temporal fluctuations in abiotic factors such as salinity, temperature, and nutrient availability. These stressful environmental conditions necessitate robust biological adaptations, making mangroves a habitat for a highly diverse microbial community, particularly bacteria and fungi (Mamangkey et al., 2021). Due to the high salinity of seawater, the resident microorganisms are predominantly halophilic or halotolerant. These adapted microbes are thus hypothesized to produce enzymes with enhanced stability and tolerance (Drissi Kaitouni et al., 2020), offering substantial potential for the bioprospecting of bacterial or fungal strains capable of yielding stable and industrially superior hydrolases.

In Buleleng Regency, Bali Province, mangrove forests are located in Gerokgak District. However, the microbial enzyme potential within these specific mangrove soils remains largely unexplored. This study, therefore, aims to isolate and identify the extracellular hydrolase-producing halophilic bacteria from mangrove soils in Sumberkima Village, Gerokgak District, Buleleng Regency. The ultimate goal is to evaluate their potential for development toward industrial-scale enzyme production.

EXPERIMENTAL SECTION

Chemicals

Chemicals such as soluble starch, carboxymethyl cellulose (CMC), KH_2PO_4, K_2HPO_4, Na_2HPO_4, NaCl, $MgSO_4$, $FeSO_4.7H_2O$, $CaCl_2$, 3,5-dinitrosalicylic acid (DNS), and rhodamine B were purchased from Merck with pro analysis grade. Yeast extract, tryptone, peptone, bacto agar, and nutrient agar were purchased from Himedia, while 4-nitrophenyl palmitate was purchased from Sigma.

Collection of Soil Samples

The soil samples were collected from the mangrove located in Sumberkima Village, Gerokgak District, Buleleng Regency, Bali Province, Indonesia (-8.13775913341742, 114.5998739699846 for SKMG1 and -8.138359208323967, 114.60031921667098 for SKMG2) (**Figure 1**). Soil samples were collected from the surface layer of the soil, at a depth of approximately 10 cm. All samples were transferred into sterile vials and immediately transported to the laboratory in an icebox for subsequent analysis.

Isolation of Halophilic Bacteria

The soil samples were diluted in sterile water containing 5% w/v NaCl, and then filtered by using sterile Whatman filter paper. The filtrates were spread on solid Luria Bertani (LB) media composed of (per liter): 10 g tryptone, 5 g yeast extract, 20 g agar, and different levels of NaCl, i.e., 50 g and 100 g. The inoculated media were subsequently incubated at 37 °C for 2 to 4 days. The bacterial colonies were then isolated to obtain pure isolates of the bacteria using fresh solid LB media.

Screening of Extracellular Hydrolase-Producing Bacteria

The extracellular amylase was investigated using starch agar (SA) media containing (per liter): 28 g nutrient agar, 10 g soluble starch, and varied NaCl content (50g and 100 g). The ability of the bacteria to produce extracellular amylase was determined by observing and measuring the diameter of the clear zone around the bacterial colonies after flooding the plates with 0.5% w/v iodine solution (Amoozegar et al., 2003).

The extracellular lipase activity was detected using rhodamine-olive oil agar (ROA) composed of (per liter): 28 g nutrient agar, 50 g or 100 g NaCl, 20 mL olive oil, and 500 μL rhodamine B solution 0.01%, pH adjusted to 7. The lipolytic potential of the bacteria was investigated by observing the orange fluorescent halos around the bacterial colonies under UV irradiation (Ameri et al., 2015).

The extracellular cellulase was determined using carboxymethyl cellulose (CMC) media containing (per liter): 28 g nutrient agar, 10 g CMC, and varied NaCl content (50 g and 100 g). The clear zone around the bacterial colony after flooding the media with a 0.5% w/v iodine solution indicates the extracellular cellulase activity of the bacteria (Naresh et al., 2019).

Figure 1. Mangrove in Sumberkima Village, Gerokgak District, Buleleng Regency, Bali Province, Indonesia.

Determination of Hydrolase Activity and Cell Dry Weight

The bacteria were inoculated into a medium containing (% w/v): nutrient broth (1.3), $MgSO_4$ (0.05), and NaCl (5 or 10). The medium was supplemented with 0.8% (w/v) of specific substrates: carboxymethyl cellulose (CMC) for cellulase, starch for amylase, and olive oil for lipase. The cultures were subsequently incubated at 37 °C for 24 hours. Extracellular enzymes were then separated from the bacterial cells via centrifugation at 8,000 × *g* for 10 minutes. The resulting bacterial pellets were dried in an oven at 80 °C for 4 hours to determine the cell dry weight (CDW). The supernatant, containing the extracellular enzymes, was collected for subsequent enzymatic activity assays.

The amylase activity was measured using DNS reducing sugar method with soluble starch as substrate (Sanjaya et al., 2024). The substrate solution was prepared by mixing 1% w/v soluble starch and 5% w/v NaCl in phosphate buffer solution pH 8. The reactions were started by adding 100 µL crude amylase into 300 µL substrate solution, incubated at 40 °C for 30 minutes. The DNS reagen (400 µL) was added into the reaction mixture and incubated at 100 °C for 5 minutes. The absorbance of the mixture was then measured at 478 nm.

The lipase activity was determined using spectrophotometric technique with 4-nitrophenyl palmitate (pNPP) as substrate (Pothayi et al., 2024). The substrate solution was prepared by mixing 2 portions of pNPP solution (12 mM) and 98 portion of buffer solution containing 0.44% w/v triton X-100 and 0.11% w/v gum arabic. The reactions were started by adding 100 µL crude lipase into 500 µL substrate solution, incubated at 40 °C for 30 minutes. The reactions were then stopped by adding 600 µL ethanol. The absorbance of the mixture was then measured at 403 nm.

The cellulase activity was measured using DNS reducing sugar method with CMC as substrate (Biswas et al., 2020). The substrate solution was prepared by mixing 0.5% w/v CMC and 5% w/v NaCl in phosphate buffer solution pH 7. The reactions were started by adding 100 µL crude cellulase into 300 µL substrate solution, incubated at 40 °C for 1 hours. The DNS reagent (400 µL) was then added into the reaction mixture and incubated in boiling water (100 °C) for 5 minutes. The absorbance of the mixture was then measured at 478 nm. The enzyme activity is expressed in unit/mL, which indicates µmol of product released by the catalytic process per minute per mL of crude enzyme.

Identification of Halophilic Bacteria

The taxonomic identity of the bacteria was determined through **16S rRNA gene analysis**. Chromosomal material was first purified from the isolates using a Geneaid Presto Mini gDNA kit. Subsequently, the 16S rRNA region was targeted for amplification via Polymerase Chain Reaction (PCR). This process utilized the primer pair Bact27F (AGAGTTTGATCATGGCTCAG) and Uni1492R (GGTTACCTTGTTACGACTT) (Parwata & Julyasih, 2025). The PCR reaction consisted of an initial denaturation at 94 °C for 4 min, followed by 34 cycles of denaturation (94 °C for 30 s), annealing (50 °C for 1 min), and extension (72 °C for 2 min). The presence and size of the PCR products were verified via agarose gel electrophoresis, followed by purification and sequencing. Once sequenced, the resulting data was refined using DNA Baser v. 3.5.4. These sequences were then compared against existing databases via the Basic Local Alignment Search Tool (BLAST) algorithm to identify closely related organisms. To conclude the study, Molecular Evolutionary Genetics Analysis (MEGA) version 5.0 was employed to perform alignment and generate a phylogenetic tree, establishing the evolutionary placement of the bacteria.

RESULTS AND DISCUSSION

More than 100 colonies of halophilic bacteria were successfully cultivated from soil samples collected at two distinct locations within the mangroves of Sumberkima Village, Gerokgak District, Buleleng Regency. A total of 32 bacterial isolates were subsequently subjected to a qualitative and quantitative assay to determine their potential for producing extracellular hydrolase enzymes (lipase, amylase, and cellulase) using specific media containing 5% and 10% w/v NaCl.

Extracellular Amylase Produced by Halophilic Bacteria

Figure 2 illustrates the extracellular amylase production among the halophilic bacteria. On SA media containing 5% w/v NaCl (**Figures 2a** and **2b**), four colonies exhibited potential for extracellular amylase production, specifically isolates SKMG1 (4, 5, 7, and 8). Conversely, in SA media with a 10% w/v NaCl (**Figures 2c** and **2d**), 13 colonies demonstrated amylase potential, comprising isolates SKMG1 (1, 2, 3, 4, 5, 7, 8) and SKMG2 (1, 2, 3, 4, 5, and 8).

The halophilic bacteria cultured in SA media with a 5% w/v NaCl content yielded a relatively low clear zone diameter (7–12 mm), with colony diameters ranging from 4–7 mm (**Table 1**). In contrast, bacteria grown on SA media containing 10% w/v NaCl produced clear zones with a significantly larger diameter (9–24 mm) while maintaining a comparable colony diameter (4–8 mm). Based on the clear zone diameter measurements, the halophilic bacteria cultured in SA media with 10% w/v NaCl exhibited higher extracellular amylase activity. Specifically, two isolates from the 10% NaCl group, SKMG2 (4) and SKMG2 (8), were identified as the most efficient extracellular amylase producers, demonstrating the largest clear zone diameters of 21 mm and 24 mm, respectively.

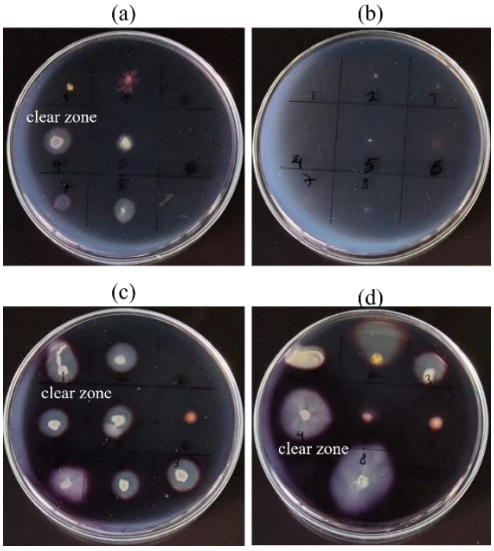

Figure 2. Amylase activity of halophilic bacteria on starch agar medium containing 5% w/v NaCl (**a** and **b**), and 10% w/v NaCl (**c** and **d**). The bacteria tested were from SKMG1 colony (**a** and **c**) and SKMG2 colony (**b** and **d**)

Tabel 1. Clear zone diameter produced by amylase activity of halophilic bacteria on starch agar medium

NaCl level (% w/v)	Bacterial colony	Clear zone diameter (mm)	Colony diameter (mm)	Bacterial colony	Clear zone diameter (mm)	Colony diameter (mm)
5	SKMG1 (4)	12	6	SKMG1 (7)	8	4
	SKMG1 (5)	7	4	SKMG1 (8)	9	7
10	SKMG1 (1)	12	4	SKMG2 (1)	11	8
	SKMG1 (2)	12	4	SKMG2 (2)	9	5
	SKMG1 (3)	12	4	SKMG2 (3)	13	4
	SKMG1 (4)	12	5	SKMG2 (4)	21	4
	SKMG1 (5)	11	5	SKMG2 (5)	6	4
	SKMG1 (7)	13	5	SKMG2 (8)	24	6
	SKMG1 (8)	11	4			

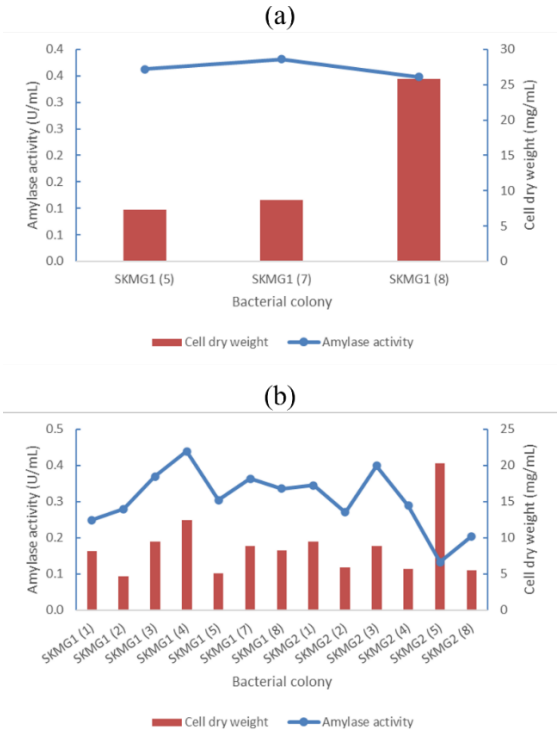

Figure 3. Extracellular amylase activity produced by halophilic bacteria. The bacteria were grown in media containing NaCl levels of 5% w/v (**a**) and 10% w/v (**b**)

Figure 3 illustrates the quantitative amylase activity produced by halophilic bacteria isolated from the Sumberkima Village mangrove soil. For the 5% NaCl growth condition (**Figure 3a**), the three bacterial isolates tested demonstrated comparable amylase activity: SKMG1 (5) yielded 0.36 U/mL; SKMG1 (7) yielded 0.38 U/mL; and SKMG1 (8) yielded 0.35 U/mL. The bacterial growth, quantified as cell dry weight (CDW), varied significantly among the isolates. Isolate SKMG1 (8) exhibited markedly superior growth, achieving a maximum biomass of 25.8 mg/mL; in contrast, isolates SKMG1 (5) and SKMG1 (7) yielded substantially lower concentrations of 7.3 mg/mL and 8.7 mg/mL, respectively. This disparity suggests that the specific productivity of isolates SKMG1 (5) and SKMG1 (7) is, in fact, substantially higher. Despite achieving significantly lower biomass (CDW), these two isolates were capable of producing amylase activity equivalent to, or marginally greater than, that of the heavily growing isolate SKMG1 (8).

For the bacterial isolates grown under 10% NaCl conditions (**Figure 3b**), 13 colonies were assayed and exhibited the capacity to produce extracellular amylase, with activities ranging from 0.13 to 0.44 U/mL. Isolate SKMG2 (5) yielded the lowest measured amylase activity (0.13 U/mL), while isolate SKMG1 (4) demonstrated the highest activity (0.44 U/mL). Additionally, isolate SKMG2 (3) also showed high amylase activity (0.40 U/mL). The data presented in **Figure 3b** suggests that, with the exception of isolate SKMG2 (5), the amylase activity produced generally correlates positively with the bacterial growth rate (CDW). Isolates characterized by high growth rates tend to produce high amylase activity, and *vice versa*. However, isolate SKMG2 (5) deviates significantly from this trend. Despite registering the lowest amylase activity, this isolate exhibited a notably higher growth rate compared to the other isolates. This finding indicates that the specific amylase productivity (activity per unit of biomass) of SKMG2 (5) is markedly low, demonstrating poor conversion efficiency relative to its level of cellular growth.

The primary amylase-producing isolate identified in this study, SKMG1 (4), exhibits high genetic homology with *Vibrio xiamenensis* (percent identity of 97.54%) (**Figure 4**). Previous studies have documented amylase production across various marine *Vibrio* species; for instance, *Vibrio* sp. has been shown to achieve optimal enzymatic activity at 55–60 °C and pH 6.5 (Najafi & Kembhavi, 2005). Similarly, *Vibrio alginolyticus* produces an amylase with peak activity at pH 6 and 60 °C, maintaining stability across a broad range of pH (5–11) and temperature (28–80 °C) (Liu et al., 2016). More recently, *Vibrio gigantis* was reported to produce extracellular amylase with an activity of 0.23 U/mL (Omeroglu et al., 2021). Notably, the amylase produced by *V. xiamenensis* SKMG1 (4) in the present study demonstrated a higher catalytic activity of 0.44 U/mL. This nearly twofold increase in activity relative to recently reported strains suggests significant potential for industrial-scale applications.

Extracellular Cellulase Produced by Halophilic Bacteria

The results of the extracellular cellulase production potential assay are illustrated in **Figure 5**. Cellulase activity was qualitatively confirmed by the visible formation of a clear zone surrounding the bacterial colonies on the assay plate. At a NaCl concentration of 5% w/v (**Figures 5a** and **5b**), 11 colonies demonstrated the capacity to produce extracellular cellulase, including isolates SKMG1 (4, 7, and 8) and isolates SKMG2 (1, 2, 3, 4, 5, 6, 7, and 8). Conversely, increasing the NaCl concentration to 10% w/v (**Figure 5c** and **5d)** resulted in 15 cellulase-producing colonies, comprising isolates SKMG1 (1, 2, 3, 4, 5, 6, 7, and 8) and isolates SKMG2 (1, 2, 4, 5, 6, 7, and 8).

The quantitative measurements of the clear zone diameter and the corresponding bacterial colony diameter for cellulase activity are presented in **Table 2**. In CMC media with 5% w/v NaCl, the bacteria produced extracellular cellulase, resulting in clear zone diameters that ranged from 17 to 30 mm (colony diameter: 4 to 15 mm). Within this NaCl concentration, two colonies, SKMG2 (3) and SKMG2 (6), exhibited the highest cellulase activity, yielding a clear zone diameter of 30 mm (colony diameters of 8 mm and 10 mm, respectively). In contrast, in CMC agar media with 10% w/v NaCl, the bacterial colonies produced extracellular cellulase with a clear zone diameter ranging from 8 to 25 mm (colony diameter: 4 to 11 mm). Based on the clear zone diameters, two isolates, SKMG1 (1) and SKMG1 (2), were identified as the most efficient producers under these high-salinity conditions, showing clear zone diameters of 24 mm and 25 mm, respectively (colony diameters of 5 mm and 6 mm, respectively).

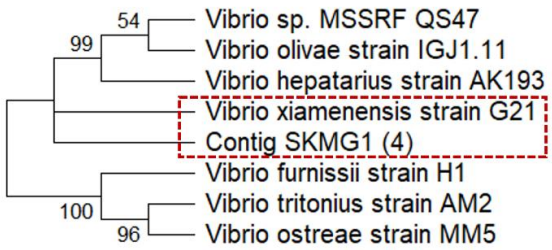

Figure 4. Phylogenetic tree of the best amylase-producing halophilic bacteria isolate SKMG1 (4)

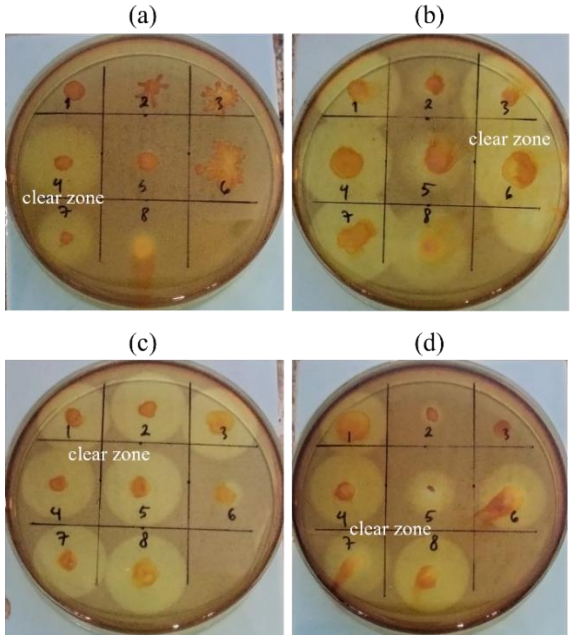

Figure 5. Cellulase activity of halophilic bacteria on CMC agar medium containing 5% w/v NaCl (**a** and **b**), and 10% w/v NaCl (**c** and **d**). The bacteria tested were from SKMG1 colony (**a** and **c**) and SKMG2 colony (**b** and **d**)

Table 2. Clear zone diameter produced by cellulase activity of halophilic bacteria on CMC agar medium

NaCl level (% w/v)	Bacterial colony	Clear zone diameter (mm)	Colony diameter (mm)	Bacterial colony	Clear zone diameter (mm)	Colony diameter (mm)
5	SKMG1 (4)	25	6	SKMG2 (4)	29	10
	SKMG1 (7)	17	4	SKMG2 (5)	21	7
	SKMG2 (1)	28	10	SKMG2 (6)	30	10
	SKMG2 (2)	15	6	SKMG2 (7)	28	9
	SKMG2 (3)	30	8	SKMG2 (8)	26	15
10	SKMG1 (1)	24	5	SKMG1 (8)	25	10
	SKMG1 (2)	25	6	SKMG2 (1)	22	11
	SKMG1 (3)	25	9	SKMG2 (4)	24	6
	SKMG1 (4)	23	6	SKMG2 (5)	14	4
	SKMG1 (5)	25	6	SKMG2 (6)	21	8
	SKMG1 (6)	8	6	SKMG2 7)	21	6
	SKMG1 (7)	23	6	SKMG2 (8)	24	7

Figure 6 presents the quantitative extracellular cellulase activity produced by the halophilic bacteria. Of the five isolates tested from the 5% w/v NaCl growth condition (**Figure 6a**), all demonstrated the capacity to produce extracellular cellulase, with activities ranging from 0.13 U/mL to 0.25 U/mL. Isolate SKMG2 (5) yielded the lowest cellulase activity (0.13 U/mL), while isolate SKMG2 (7) produced the highest activity (0.25 U/mL). The data reveals that the bacterial growth rate (CDW) is inversely proportional to the cellulase activity produced. For instance, two isolates, SKMG2 (5) and SKMG2 (8), which achieved very high growth rates (CDW 55 and 50 mg/mL, respectively), yielded the lowest cellulase activity (0.13 U/mL). Conversely, isolates SKMG2 (6) and SKMG2 (7), with significantly lower growth rates (CDW 2.9 and 5.2 mg/mL), were capable of producing high cellulase activity, at 0.23 U/mL and 0.25 U/mL, respectively. This finding strongly suggests that the specific productivity (enzyme yield per unit of biomass) of isolates SKMG2 (6) and SKMG2 (7) is substantially higher than that of the other isolates.

For the 10% NaCl isolates, 14 tested colonies were capable of producing extracellular cellulase, with activities ranging from 0.09 U/mL to 0.36 U/mL (**Figure 6b**). Isolates SKMG1 (6) and SKMG2 (5) were identified as the lowest cellulase producers, both yielding an activity of 0.09 U/mL. In contrast, the highest cellulase activity (0.36 U/mL) was produced by two isolates: SKMG1 (1) and SKMG1 (3). Similar to the 5% NaCl condition, bacterial growth was generally uncorrelated with the measured cellulase activity. Isolates characterized by high growth rates (CDW), such as SKMG1 (6), SKMG2 (5), SKMG2 (6), and SKMG2 (7), produced only low cellulase activity. Conversely, isolates with lower biomass accumulation

were able to produce high cellulase activity, further supporting the observation that enzyme production is decoupled from biomass accumulation under these conditions.

The most proficient cellulase-producing isolate, SKMG1 (3), exhibits close homology with *Vibrio hepatarius* (percent identity 97.90%) (**Figure 7**). Various cellulase-producing bacteria have been documented within genera such as *Bacillus*, *Brevibacterium*, *Exiguobacterium*, *Halomonas*, *Sanguibacter*, and *Staphylococcus* (Drissi Kaitouni et al., 2020). One notable study identified cellulase activity in a *Vibrio* sp. isolated from soil at the Shandong University campus, China (Li et al., 2003). Given its cellulase activity of 0.36 U/mL, this bacterium holds significant potential for development as a large-scale cellulase producer.

Extracellular Lipase Produced by Halophilic Bacteria

The potential for extracellular lipase production from the halophilic bacterial isolates is illustrated in **Figure 8**. Lipase activity was qualitatively assessed by the presence of fluorescence surrounding the bacterial colonies, indicating the hydrolysis of the assay substrate. At a NaCl concentration of 5% w/v (**Figure 8a** and **8b**), 10 bacterial colonies demonstrated the potential to produce extracellular lipase, specifically isolates SKMG1 (2, 3, 6, and 8) and isolates SKMG2 (1, 2, 3, 5, 7, and 8). A higher number of isolates producing extracellular lipase were observed at a NaCl concentration of 10% w/v (Figure 8c and 8d), totaling 13 colonies, which included isolates SKMG1 (1, 3, 4, 6, 7, and 8) and isolates SKMG2 (2, 3, 4, 5, 6, 7, and 8).

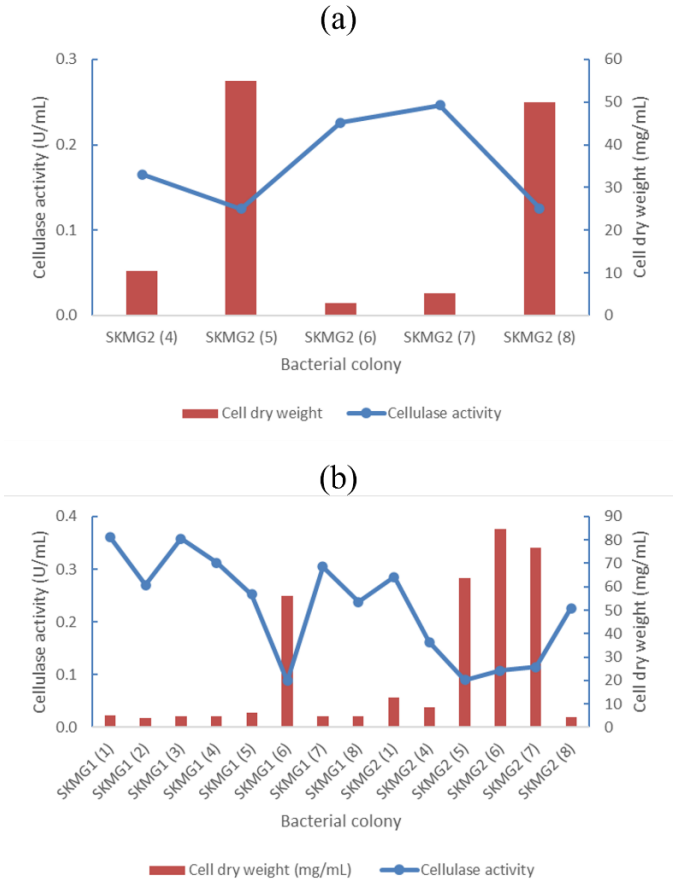

Figure 6. Extracellular cellulase activity produced by halophilic bacteria. The bacteria were grown under NaCl levels of 5% w/v (**a**) and 10% w/v (**b**)

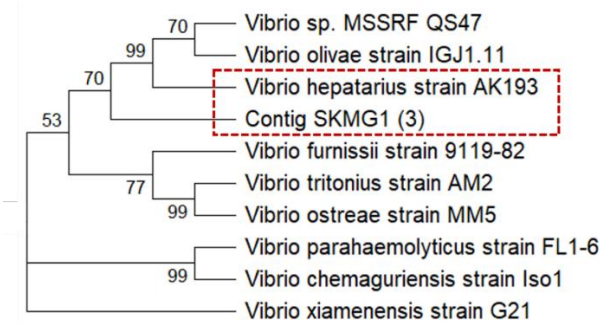

Figure 7. Filogenetic three of the best cellulase-producing halophilic bacteria isolate SKMG1 (3)

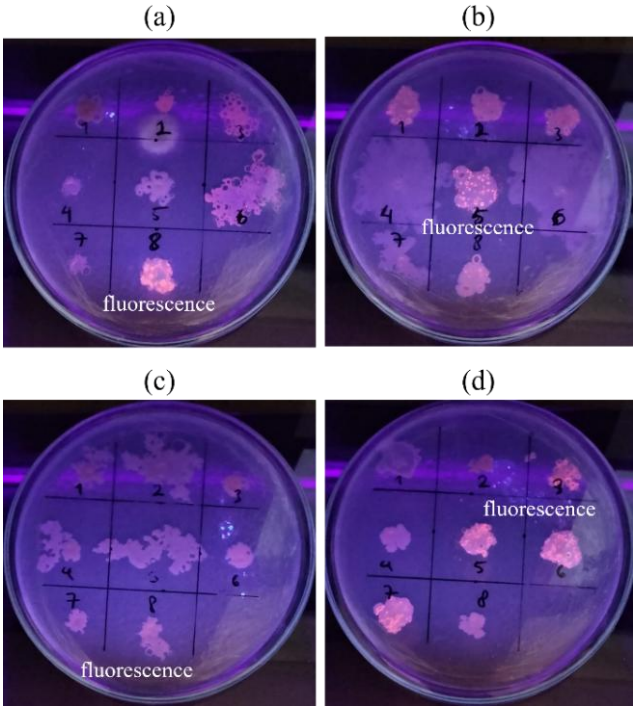

Figure 8. Lipase activity of halophilic bacteria on rodhamine olive oil agar medium containing 5% w/v NaCl (**a** and **b**), and 10% w/v NaCl (**c** and **d**). The bacteria tested were from SKMG1 colony (**a** and **c**) and SKMG2 colony (**b** and **d**)

Based on the data derived from the qualitative fluorescence intensity measurements (**Table 3**), bacterial colonies grown in media with a NaCl concentration of 5% w/v exhibited a fluorescence intensity ranging from 4 to 8. In contrast, bacterial colonies from the media containing 10% w/v NaCl showed a fluorescence intensity range of 2 to 8. Analyzing the colony fluorescence intensity data, two bacterial isolates grown at 5% w/v NaCl were identified as the most active lipase producers: SKMG1 (8) and SKMG2 (5), both displaying the maximum fluorescence intensity value of 8. Conversely, in media with a NaCl concentration of 10% w/v, three isolates were among the best lipase producers, also achieving a fluorescence intensity value of 8: SKMG2 (5, 6, and 7).

Figure 9 displays the quantitative extracellular lipase activity produced by the halophilic bacterial isolates from the Sumberkima mangrove soil. For the 5% NaCl growth condition (**Figure 9a**), isolates demonstrated extracellular lipase activity ranging widely from 0.4 U/mL to 21.6 U/mL. Three isolates SKMG1 (2), SKMG1 (3), and SKMG1 (6) were identified as the lowest lipase producers, yielding 0.4, 0.7, and 0.5 U/mL, respectively. Isolates SKMG2 (1), SKMG2 (7), SKMG1 (8), and SKMG2 (8) produced higher activities, ranging from 3.5 U/mL to 9.8 U/mL. Notably, isolate SKMG2 (5) was the most potent lipase producer, achieving the maximum recorded activity of 21.6 U/mL. A lack of proportionality was observed between bacterial growth and the enzyme activity produced. Isolate SKMG2 (5), despite having a lower growth rate compared to isolates SKMG2 (1, 3, 7, and 8), produced significantly higher lipase activity than those four isolates.

The assay results for the 10% NaCl growth condition (**Figure 9b**) indicated lipase activity varying from 0.2 U/mL to 13.5 U/mL. The three lipase-producing isolates with the lowest activities (0.2 to 0.3 U/mL) were SKMG2 isolates (2, 5, and 8). Conversely, three other isolates (SKMG1 (8), SKMG1 (3), and SKMG2 (4)) yielded higher activities of 1.0, 1.1, and 1.6 U/mL, respectively. Two isolates, SKMG2 (6) and SKMG2 (7), were the most active lipase producers under this high-salinity condition, with activities of 12.9 U/mL and 13.5 U/mL. Bacterial growth also remained uncorrelated with the measured lipase activity. Specifically, isolate SKMG2 (4), which registered high growth (only slightly lower than the most active producers, SKMG2 (6) and SKMG2 (7)), produced lipase activity that was significantly lower than that of the two best isolates. This reinforces the conclusion that specific enzyme productivity is highly isolate-dependent and decoupled from total biomass accumulation.

The most potent lipase-producing isolate, SKMG2 (5), demonstrates close homology with Salinivibrio kushneri (percent identity 98.39%) (**Figure 10**). Lipase activity has been documented in various bacteria isolated from mangrove ecosystems, including the genera Pseudomonas, Acinetobacter, Alteromonas, Alcaligenes, Beijerinckia, Acetobacter, Proteus, Derxia, Flavobacterium, and Bacillus (Varghese et al., 2021). Notably, a study of a Salinivibrio sp. isolated

Table 3. Fluorescence intensity produced by lipase activity of halophilic bacteria on rodhamine olive oil agar medium (assessment was carried out qualitatively on a scale of 0-10)

NaCl level (% w/v)	Bacterial colony	Fluorescence intensity	Bacterial colony	Fluorescence intensity
5	SKMG1 (2)	6	SKMG2 (2)	6
	SKMG1 (3)	6	SKMG2 (3)	6
	SKMG1 (6)	6	SKMG2 (5)	8
	SKMG1 (8)	8	SKMG2 (7)	4
	SKMG2 (1)	6	SKMG2 (8)	6
10	SKMG1 (1)	4	SKMG2 (3)	6
	SKMG1 (3)	6	SKMG2 (4)	4
	SKMG1 (4)	2	SKMG2 (5)	8
	SKMG1 (6)	6	SKMG2 (6)	8
	SKMG1 (7)	4	SKMG2 (7)	8
	SKMG1 (8)	6	SKMG2 (8)	4
	SKMG2 (2)	2		

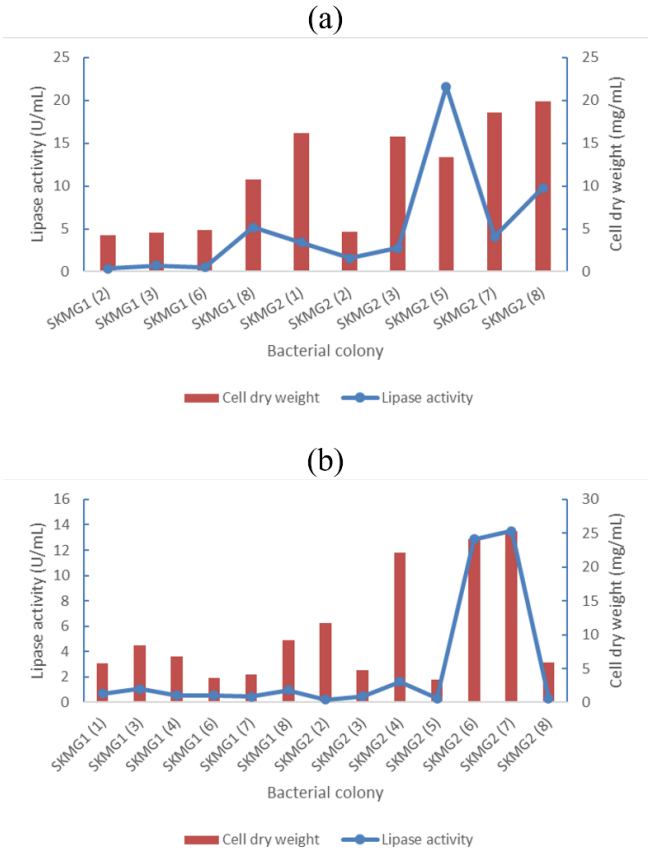

Figure 9. Extracellular lipase activity produced by halophilic bacteria. The bacteria were grown under NaCl levels of 5% w/v (**a**) and 10% w/v (**b**)

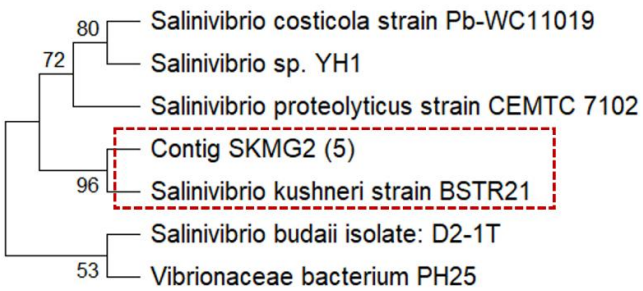

Figure 10. Phylogenetic tree of the best cellulase-producing halophilic bacteria isolate SKMG2 (5)

from a hypersaline ecosystem in Iran identified a lipase with robust thermal tolerance up to 80 °C (Amoozegar et al., 2008). In the present study, the initial lipase activity recorded for Salinivibrio kushneri SKMG2 (5) was remarkably high (21.6 U/mL). This substantial baseline activity suggests that the strain holds significant promise for industrial-scale production, particularly if yields are further enhanced through the optimization of media and fermentation conditions.

CONCLUSIONS

This study successfully isolated several halophilic bacteria capable of producing extracellular hydrolases from mangrove soil in Sumberkima Village, Gerokgak, Buleleng, Bali, Indonesia. A total of 17 amylase-producing isolates were identified with activities ranging from 0.13 to 0.44 U/mL, alongside 24 cellulase-producing isolates (0.09–0.36 U/mL) and 23 lipase-producing isolates (0.2–21.6 U/mL). Taxonomic identification revealed the most proficient producers as *Vibrio xiamenensis* SKMG1 (4) for amylase, *Vibrio hepatarius* SKMG1 (3) for cellulase, and *Salinivibrio kushneri* SKMG2 (5) for lipase. Notably, this research constitutes the first report of hydrolase production from these three specific bacterial species.

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