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Diversity of seaweed-derived marine bacteria producing sulfated polysaccharide-degrading enzymes: Isolation, Screening, and Identification

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ABSTRACT

Marine bacteria associated with algae represent a valuable source of enzymes capable of degrading complex polysaccharides, such as fucoidan and ulvan. This study investigated bacteria isolated from 12 species of brown algae and 8 species of green algae, yielding 126 and 65 strains, respectively. Selective media supplemented with fucoidan or ulvan as the sole carbon source was employed to screen for enzymatic activity. The bacterial strains were characterized based on morphological features, and molecular identification was conducted through 16S rRNA sequencing. Several isolates demonstrated significant activity on fucoidan from *Sargassum mcclurei* and ulvan from *Ulva fasciata*. Phylogenetic analysis revealed a wide taxonomic range, with strains classified into the genera *Alteromonas, Bacillus, Vibrio, Cobetia, and Delftia.* This diversity underscores the metabolic adaptability of marine bacteria in degrading sulfated polysaccharides. The results provide insights into the enzymatic potential of marine bacteria for biotechnological applications, particularly in the conversion of algal biomass into bioactive compounds. These findings lay the foundation for further studies on enzyme characterization and industrial utilization of marine-derived polysaccharide-degrading bacteria.

Keywords: Fucoidan, ulvan, sulfated polysaccharide-degrading enzymes, marine bacteria.

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INTRODUCTION

Fucoidan and ulvan are sulfated polysaccharides found in the cell walls of brown algae (*Phaeophyceae*) and green algae (*Chlorophyta*), such as *Ulva* species [1, 2]. Fucoidan, composed primarily of fucose and sulfate groups, and ulvan, rich in rhamnose, glucuronic acid, and sulfate, exhibit significant biological activities, including antioxidant, anti-inflammatory, and antiviral effects [3–5]. These properties make them valuable for pharmaceutical and nutraceutical applications [6, 7]. However, their structural complexity necessitates enzymatic degradation to unlock their full biotechnological potential.

Enzymes such as fucoidanase and ulvan lyase are pivotal in this process. Fucoidanase hydrolyzes fucoidan into bioactive oligosaccharides [8], while ulvan lyase specifically cleaves ulvan’s glycosidic bonds to produce functional oligosaccharides [9]. These enzymes facilitate the study of the structure-function relationships of sulfated polysaccharides and enable the production of high-value products for industrial applications [10, 11]. The discovery and characterization of these enzymes are, therefore, crucial for advancing the utilization of these marine resources.

Marine ecosystems are known for their extraordinary biodiversity and host various microorganisms, including bacteria capable of producing enzymes adapted to extreme environmental conditions [12]. Among these, seaweed-associated bacteria are particularly promising as they have evolved to utilize seaweed-derived polysaccharides as carbon and energy sources. These bacteria often produce specialized enzymatic systems, such as fucoidanase, alginate lyase, and ulvan lyase to degrade the complex structures of sulfated polysaccharides [13, 14]. The metabolic diversity and adaptability of these microorganisms represent a valuable reservoir of enzymes with significant biotechnological potential, warranting systematic efforts to isolate and characterize such bacteria.

Seaweed habitats, including brown and green algae, are abundant in tropical coastal waters, such as those of Vietnam, home to a rich diversity of seaweed species. Brown algae (*Sargassum*, *Padina*, and *Turbinaria*) and green algae (*Ulva* and *Caulerpa*) dominate in these ecosystems, particularly along the central and southern coasts of Vietnam, including areas like Nha Trang Bay [15, 16]. These algae provide ecological niches for marine bacteria, many forming symbiotic associations [17]. These symbionts are potential sources of enzymes capable of breaking down the sulfated polysaccharides found in their algal hosts.

This study isolates and tests marine bacteria from seaweed samples collected in Nhatrang Bay to investigate their capacity to degrade sulfated polysaccharides, such as fucoidan and ulvan. This approach aims to explore the diversity of seaweed-associated bacteria and identify strains with enzymatic potential, paving the way for further studies on enzyme characterization and their industrial applications.

MATERIALS AND METHODS

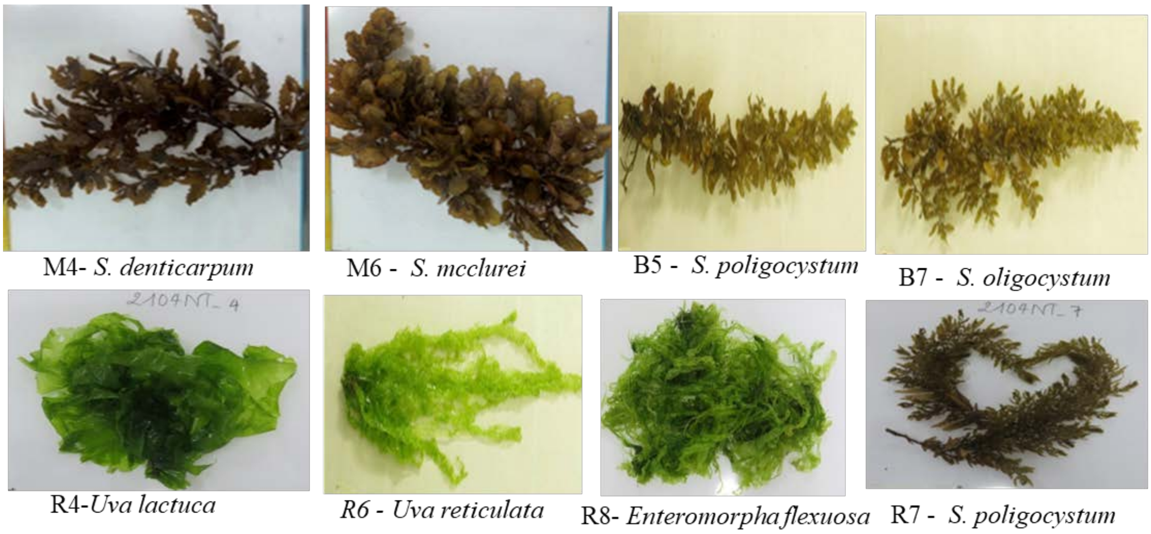
Materials

Fucoidan from *Sargassum mcclurei* and ulvan from *Ulva fasciata* were extracted following the method described by Pham Duc Thinh et al., [18] and Cao Thi Thuy Hang et al., [19] and were provided by the Analytical Chemistry and Technology Development Laboratory.

Methods

Sampling and sample treatment

A total of 12 brown algae and 8 green algae samples were collected from the coastal waters of Nha Trang, Khánh Hòa province, at a depth of 3–5 meters on April 21, 2022, May 2022, and April 2023 (Fig. 1). The collected samples were immediately stored in sterile ziplock bags, maintained under chilled conditions, and transported to the laboratory for processing. Under sterile conditions, the algal samples were submerged in 100 mL of sterile seawater supplemented with 5% peptone but lacking any carbon source. The samples were incubated for 7–15 days until visible degradation of the algae was observed. The resulting solution was then utilized to isolate marine bacteria.



M4- *S. denticarpum* M6 - *S. mcclurei* B5 - *S. poligocystum* B7 - *S. oligocystum*

R4-*Uva lactuca* *R6 - Uva reticulata* R8- *Enteromorpha flexuosa* R7 - *S. poligocystum*

Fgure 1. Brown and green algae samples collected from the coastal waters of Khanh Hoa

Isolation of marine bacteria

Marine bacteria were isolated using a selective medium with the following composition (per liter): 5 g peptone, 28 g sea salts, and 15 g agar, adjusted to a pH of 7.4–7.8. The medium was supplemented with 1% fucoidan (MBF) or 1% ulvan (MBU) as the carbon source. A 100 µL aliquot of the degraded seaweed solution was serially diluted and spread onto agar plates containing MBF or MBU medium. The plates were incubated at 30°C for 3–7 days or until bacterial colonies appeared. Individual colonies were transferred to a liquid Marine Broth (MB) medium to obtain pure cultures. The bacterial colonies were examined for morphological characteristics, assigned unique codes, and preserved in a liquid MB medium supplemented with 30% glycerol (v/v) at -80°C. The isolates were stored in the Marine Microbial Collection (NCMM) at the Institute of Research and Application of Technology Nha Trang (NITRA).

Screening for sulfated polysaccharide-degrading enzyme activity

Purified bacterial strains were inoculated onto MBF and MBU agar plates containing fucoidan or ulvan as the sole carbon source and incubated at 30°C for 3–5 days. After incubation, the agar surface was washed with distilled water to remove biomass. Enzyme activity was assessed by staining with 1% (w/v) hexadecyltrimethylammonium bromide (Cetavlon) for 30 minutes at room temperature. Clear zones around colonies indicated polysaccharide hydrolysis, confirming fucoidanase or ulvan lyase activity, while non-degraded regions formed an opaque white precipitate [20].

Identification of selected marine bacteria

For molecular identification, genomic DNA was extracted using the NucleoSpin DNA extraction kit (Clontech, CA, USA) according to the manufacturer’s instructions. The 16S rRNA gene was amplified using primers 533F (5’-GTGCCAGCAGCCGCGGTAA-3’) and 1496R (5’-GGTTACCTTGTTACGACTT-3’), targeting a ~1.5 kb region. PCR reactions (25 µL) included 12.5 µL CloneAmp polymerase mix, 1 µL of each primer (0.5 µM), 1 µL DNA template (10 ng/µL), and 9.5 µL sterile deionized water. Thermal cycling conditions were: initial denaturation at 98°C for 30 seconds, followed by 20 cycles of 98°C for 10 seconds, 58°C for 10 seconds, 72°C for 1 minute, with a final extension at 72°C for 10 minutes, and hold at 8°C. PCR products were analyzed on a 2% agarose gel, purified using the QIAquick PCR Purification Kit (Qiagen, Germany), and sequenced by the Sanger method using an ABI 3130XL Genetic Analyzer (Thermo Fisher Scientific, USA) at Nam Khoa Trading and Service Co., Ltd. Laboratory (Ho Chi Minh City, Vietnam).

Phylogenetic tree construction

The 16S rRNA gene sequences of selected bacterial strains were aligned against the NCBI nucleotide database (nr/nt) using BLAST to identify closely related sequences. Multiple sequence alignment was performed using the ClustalW algorithm in MEGA X [21] with default parameters. The aligned sequences were trimmed at both ends and concatenated using MEGA X and BioEdit 7.2. Manual adjustments were made to remove ambiguities and improve alignment quality. A Maximum Likelihood (ML) phylogenetic tree was constructed in MEGA X using the best-fit substitution model determined by the software. Bootstrap analysis with 1,000 replicates was performed to assess the tree's reliability. The resulting phylogenetic tree was visualized using the Chiplot online platform [22].

RESULTS AND DISCUSSTION

Isolation and screening of marine isolates for polysaccharide-degrading enzymes

A total of 126 bacterial strains were isolated from 12 species of brown algae, and 65 strains were obtained from 8 species of green algae. The isolates exhibited diverse colony morphologies, including color, shape, and size variations. These differences suggest that the isolates represent distinct bacterial strains, preserved for subsequent screening of polysaccharide-degrading enzyme activity (Fig. 2). All isolates grew on selective media containing fucoidan or ulvan as the sole carbon source, indicating a high potential for producing enzymes capable of degrading these sulfated polysaccharides.

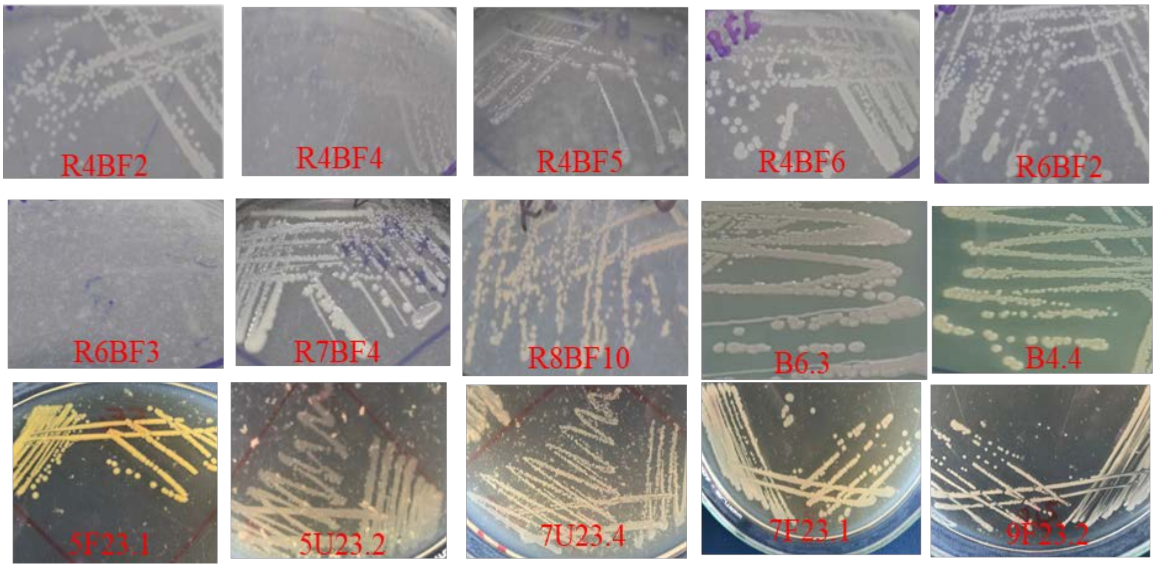
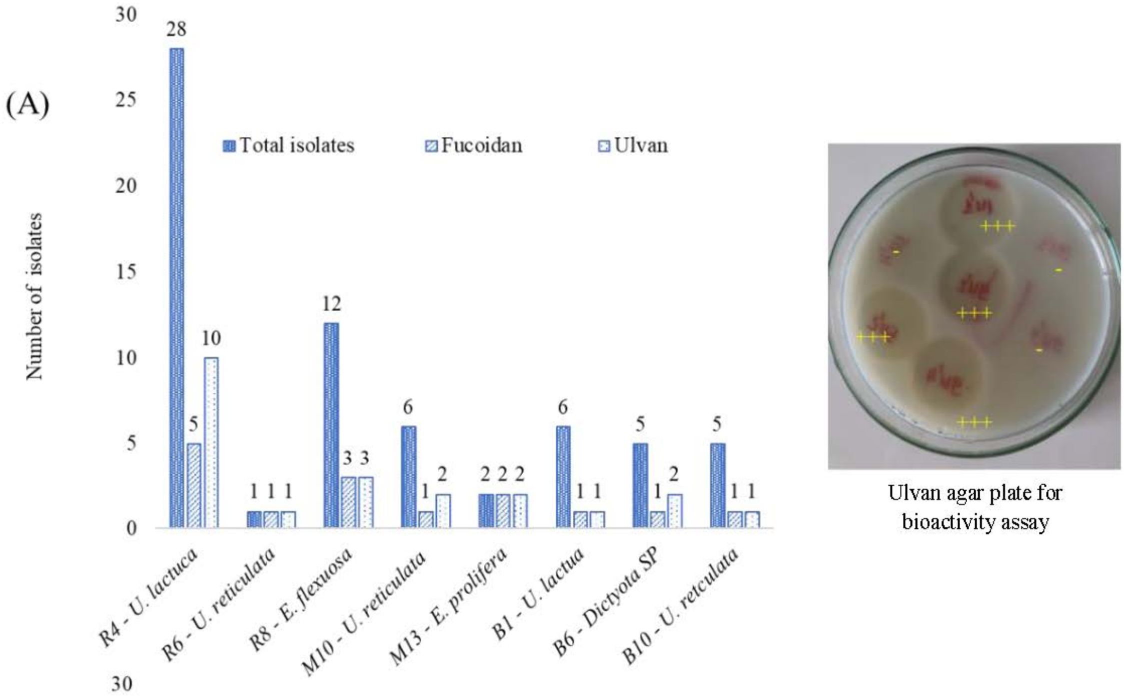
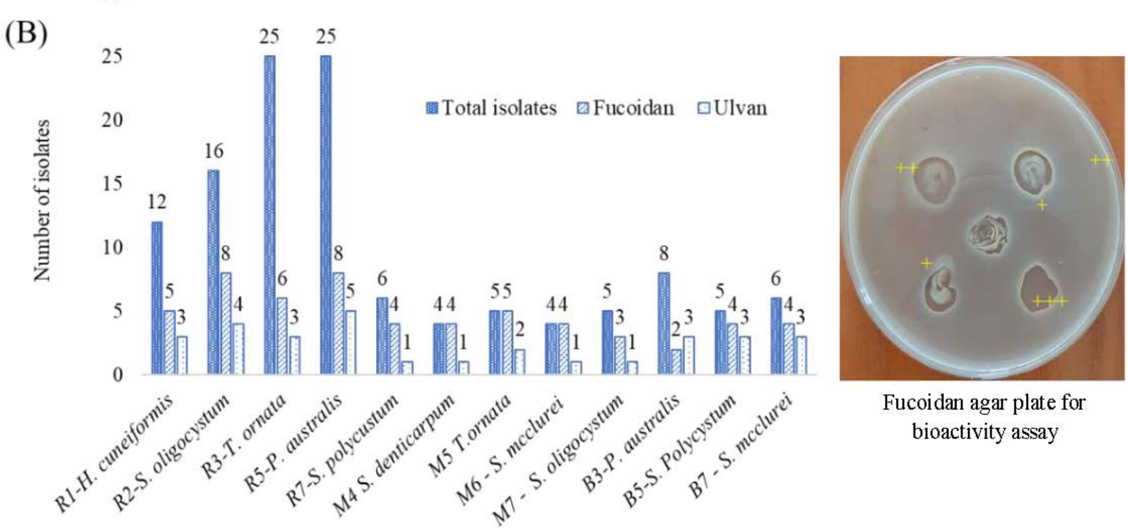


Figure 2. Colony morphology of selected isolated bacterial strains

The enzymatic activity of bacterial strains isolated from green and brown algae shows notable variations across different algal sources (Fig. 3). Among 134 strains from green algae, R4 - *U. lactuca* yielded the highest number of isolates (28), with 5 strains active on fucoidan and 10 on ulvan, while R8 - *E. flexuosa* and M10 - *U. reticulata* exhibited moderate activity (1–3 strains). Despite fewer isolates, R6 - *U. reticulata* and B10 - *U. reticulata* showed balanced activity on both substrates, indicating functional specialization.



Ulvan agar plate for bioactivity assay



Fucoidan agar plate for bioactivity assay

Figure 3. The number of bacterial strains isolated from algae and their enzymatic activity on ulvan and fucoidan agar plates: (A) Green algae and (B) Brown algae

From brown algae, 126 strains were isolated, with R3 - *T. ornata* and R5 - *P. australis* producing the most isolates (25 each) and R5 showing significant enzymatic activity (8 on fucoidan, 5 on ulvan). R2 - *S. oligocystum* strains also showed strong potential, while R1 - *H. cuneiformis* and R7 - *S. polycustum* displayed moderate activity. Notably, marine-derived isolates (e.g., M4 - *S. denticarpum*, M6 - *S. mcclurei*) exhibited high specificity for fucoidan, and isolates from B7 - *S. mcclurei* and B9 - *S. olygocystum* demonstrated balanced activity on both substrates.

The discrepancy between the high number of bacterial isolates and the fewer strains showing enzymatic activity on both fucoidan and ulvan substrates may result from suboptimal growth conditions in the selective medium. Factors such as pH, temperature, and salinity could be unsuitable for some strains, reducing growth and enzyme production [1]. Additionally, certain enzymes may remain intracellular and require specific extraction methods to be detected [8]. Optimizing growth conditions and employing proper enzyme extraction techniques are crucial for accurately assessing the enzymatic potential of these isolates.

Other marine organisms as valuable sources of polysaccharide-degrading enzymes include bacterial strains isolated from sponges and seaweeds in tropical coastal waters have demonstrated significant alginate lyase and fucoidanase activity, highlighting their ability to degrade complex polysaccharides like fucoidan and alginate into bioactive oligosaccharides. Research by Nguyen et al., (2019) and Vo et al., (2020) successfully isolated bacterial strains from the Vietnamese marine environment, which showed high activity on brown seaweed polysaccharides such as alginate and fucoidan. These studies established the potential of marine bacteria in producing enzymes like fucoidanase and alginate lyase and contributed significantly to understanding their ecological roles.

Identification and classification of selected marine bacterial strains

16S rDNA sequencing identified bacterial strains with high activity on fucoidan and ulvan and their sequences were deposited in the NCBI GenBank database (Table 1).

Table 1. Identification, sources, and enzymatic activites of selected marine bacterial strains

No.	Code	Sources of Isolation	Gene code	Identification	Substrate	
					Fucoidan	Ulvan
1	R4BF2	R4- Green algae *Ulva lactuca*	PQ643436	*Alteromonas alvinellae* NCMM-KB1	+++	+++
2	R4BF4		PQ643437	*Bacillus velezensis* NCMM-KB2	++	+++
3	R4BF5		PQ643438	*Vibrio xiamenensis* NCMM-KB3	++	+++
4	R4BF6		PQ643439	*Alteromonas macleodii* NCMM-KB4	+++	++
5	R6BF2	R6 - Green algae *Ulva reticulata*	PQ643440	*Bacillus altitudinis* NCMM-KB5	++	++
6	R6BF3		PQ643441	*Alteromonas macleodii* NCMM-KB6	+++	+
7	R7BF4	R7 - Brown algae *S. polycustum*	PQ643442	*Cobetia amphilecti* NCMM-KB7	+++	+++
8	R8BF10	R8 - Green algae *Enteromorpha flexuosa*	PQ643443	*Bacillus velezensis* NCMM-KB8	+	+
9	M6.3	M6 - *S. mcclurei*	PQ643444	*Delftia tsuruhatensis* NCMM-KB9	+++	+++
10	5F23.1	B5 - *S. poligocystum*	PQ643454	*Alteromonas macleodii* NCMM-KB10	++	++
11	5U23.2		PQ643455	*Alteromonas macleodii* NCMM-KB11	++	++
12	7U23.4	B7 - *S. ooligocystum*	PQ643456	*Alteromonas confluentis* NCMM-KB12	++	+++

Note: "+": low enzymatic activity; "++": moderate enzymatic activity and "+++": high enzymatic activity on studies substrates.

The enzymatic activity of the selected bacterial strains on fucoidan and ulvan substrates strongly correlates with their algal sources. Strains from green algae (*U.lactuca*, *U. reticulata*, *Enteromorpha flexuosa*) and brown algae (*Sargassum polycystum*, *S. oligocystum*) exhibited diverse activity levels, reflecting ecological adaptation to their hosts' polysaccharide compositions. High activity on both substrates was observed in R4BF2 and R7BF4 from *U. lactuca* and *S. polycystum*, indicating broad enzymatic potential. Moderate to high activity in R4BF6, 5F23.1, and M6.3 suggests specialized enzymatic systems. Conversely, R8BF10 displayed low activity, likely due to narrower specialization or low polysaccharide content. Strains from *S. polycystum* and *S. oligocystum* (e.g., 5F23.1, 7U23.4) showed balanced activity, reflecting adaptation to diverse polysaccharides in brown algae.

The phylogenetic tree, constructed from 16S rDNA sequences, illustrates the evolutionary relationships among the selected marine bacterial strains exhibiting high enzymatic activity against sulfated polysaccharides. Specifically, the analysis includes fucoidanase enzymes classified in glycoside hydrolase families GH107, GH168, GH174, and GH187, as well as ulvan lyases grouped in polysaccharide lyase families PL24, PL25, PL28, and PL40 (Fig. 4).

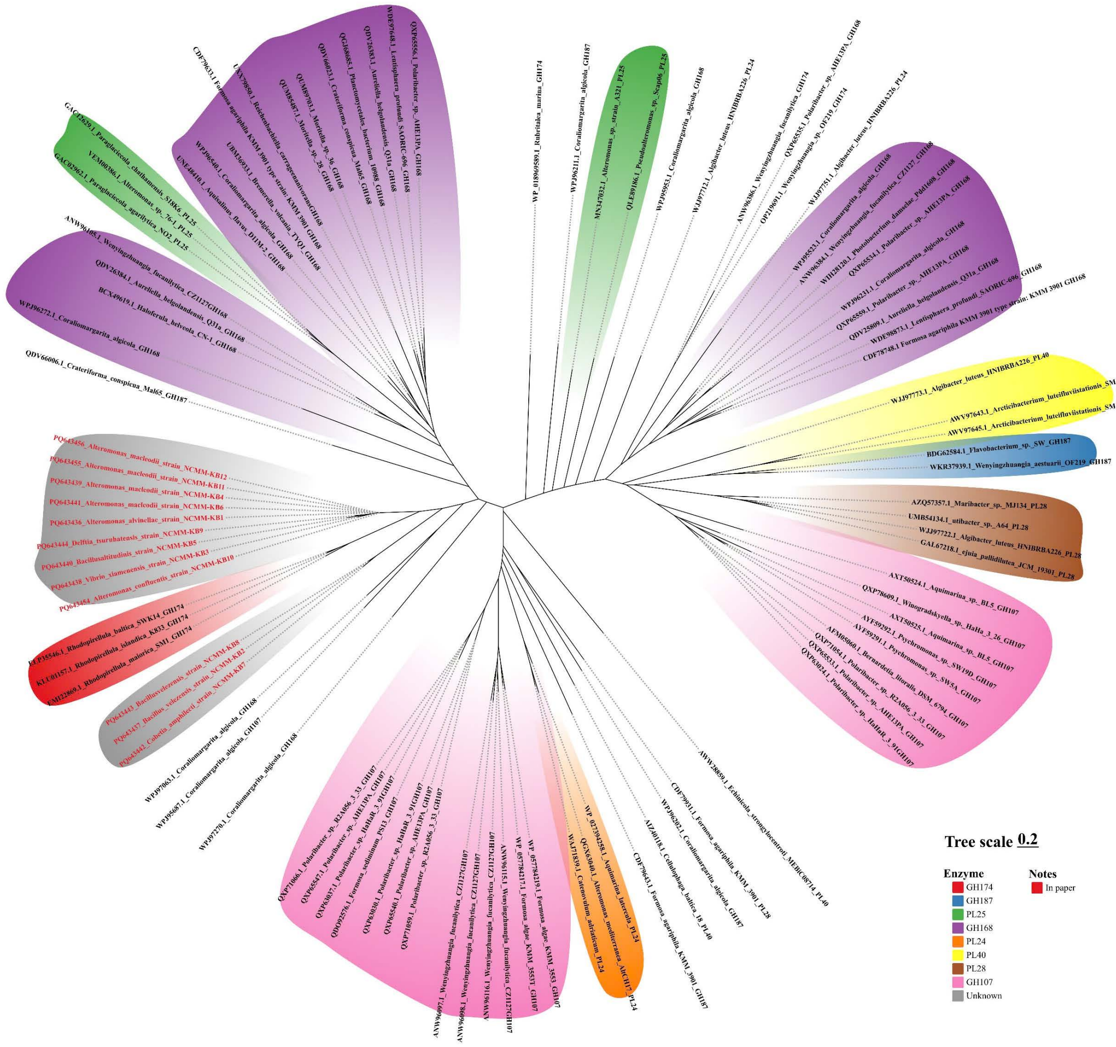


Figure 4. Phylogenetic tree of selected marine bacterial strains based on 16S rDNA sequences and their associated enzymatic activities

Strains marked in red within the phylogenetic tree are closely associated with enzymes involved in the hydrolysis of sulfated polysaccharides, specifically fucoidanase (GH107, GH168, GH174, GH187) and ulvan lyase (PL24, PL25, PL28, PL40). Notably, strains from the genera *Alteromonas* and *Bacillus*—including *A. macleodii* and *B. velezensis*—are near branches linked to these enzymes, indicating their strong potential to degrade seaweed-derived polysaccharides. This aligns with their ecological adaptation, wherein specialized enzymatic systems are developed to exploit sulfated polysaccharides as energy sources.

Fucoidanase enzymes (GH107, GH174) and ulvan lyases (PL24, PL28) play essential roles in cleaving specific glycosidic bonds, generating bioactive oligosaccharides. Strains of *Alteromonas*, prominently distributed among the enzyme-related branches, exhibit remarkable capabilities for targeting both fucoidan and ulvan, highlighting their biotechnological potential.

CONCLUTION

This study highlights seaweed-associated marine bacteria's significant diversity and enzymatic potential of in Nha Trang Bay, Vietnam. From a total of 191 bacterial strains isolated, several demonstrated high enzymatic activity, particularly against fucoidan and ulvan, emphasizing their ecological role and biotechnological relevance. Molecular identification linked these strains to genera such as *Alteromonas, Bacillus, Vibrio, Cobetia,* and *Delftia*, all known for their polysaccharide-degrading capabilities. Phylogenetic analysis further confirmed their close relationships with enzymes like fucoidanase and ulvan lyase, which are critical for converting complex sulfated polysaccharides into bioactive oligosaccharides. The findings not only enrich our understanding of tropical marine microbial resources but also underline the immense potential of these bacteria for industrial applications, including pharmaceuticals, nutraceuticals, and biofuel production. Future research should focus on characterizing the enzymatic mechanisms, optimizing their activity under industrial conditions, and evaluating the bioactivity of the resulting oligosaccharides.

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