

Exiguobacterium sargassi sp. nov., a novel seaweed-associated bacterium isolated from Sargassum henslowianum with a glycoside-hydrolase-enriched CAZyme repertoire

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Abstract

Marine macroalgae are rich in complex polysaccharides, making them ideal habitats for finding undiscovered bacterial lineages with unique metabolic capabilities. A novel Gram-variable, facultatively anaerobic bacterium, designated strain RM11^T, was isolated from the internal tissue of *Sargassum henslowianum* collected in Quang Ngai province, Vietnam. Growth occurred at 10–45°C, pH 7.0–10.0 and 1–12% (w/v) NaCl. Phylogenetic analysis of the 16S rRNA gene placed strain RM11^T within the genus *Exiguobacterium*, with the highest sequence similarity to *E. qingdaonense* S82^T (99.82%). Average nucleotide identity (ANI) values ranged from 86.46 to 90.92% and digital DNA–DNA hybridization (dDDH) values from 30.5 to 41.7%, both well below species-delineation thresholds (95–96% ANI; 70% dDDH). The genome (2,859,837 bp; G + C 47.79%) encodes genes for heavy-metal transport, ammonia assimilation, and oxidative and osmotic stress responses. Annotation of carbohydrate-active enzymes (CAZymes) revealed a glycoside-hydrolase-dominated repertoire enriched in GH13 family members predicted to participate in α -glucan metabolism, together with a candidate secreted GH16_3–CBM4 β -glucanase, whereas no polysaccharide lyase genes were detected. Based on the combined phenotypic, chemotaxonomic and genomic evidence, we propose *Exiguobacterium sargassi* sp. nov., with the type strain RM11^T (= KCTC 43930^T = InaCC B1791^T).

1. Introduction

Brown macroalgae (Phaeophyceae) including commercially important species such as *Sargassum* spp., are foundational components of marine ecosystems characterized by their rich and diverse carbohydrate composition. Their complex carbohydrate matrices comprise structural polysaccharides such as alginate and fucoidan, as well as intracellular storage compounds including the polysaccharide laminarin and the sugar alcohol mannitol, thereby providing unique ecological niches for diverse seaweed-associated microorganisms. These endophytic and epiphytic marine bacteria possess remarkable enzymatic capabilities, secreting carbohydrate-active enzymes (CAZymes) capable of depolymerizing both structural and storage polysaccharides into oligosaccharides and monomeric sugars for survival and growth. The enzymatic depolymerization of seaweed polysaccharides has emerged as a major frontier in biotechnology, with far-reaching implications for industrial applications and human health¹.

Brown macroalgal polysaccharides and their associated microbial communities hold tremendous promise as precursors for bioactive compounds and functional ingredients with diverse applications. Alginate, distinguished by its ionotropic gel-forming capacity in the presence of divalent cations, high aqueous viscosity, and biocompatibility, is already widely utilized in the food industry as a thickener, gelling agent, emulsion stabilizer, and microencapsulation matrix^{2,3}. Beyond structural polymers, the intracellular storage polysaccharides—predominantly laminarin (β -1,3-glucan with occasional β -1,6 branches)—represent a largely untapped resource for generating bioactive oligosaccharides. These oligosaccharides have demonstrated significant potential as functional food ingredients, with preclinical studies suggesting beneficial effects on gut microbial composition, inflammatory responses, oxidative stress markers, and lipid metabolism. However, current evidence is primarily derived from in vitro and animal experiments, underscoring the need for systematic identification of novel CAZymes with unique substrate specificities and catalytic properties^{4,5}.

In recent years, the enzymatic depolymerization of seaweed polysaccharides has attracted significant biotechnological interest. While much research has traditionally focused on alginate lyases, the degradation of α - and β -glucans by marine bacteria is equally critical. Enzymes such as β -glucanases and various glycoside hydrolases (GHs) targeting complex carbohydrates are essential for generating bioactive oligosaccharides. These oligosaccharides have demonstrated potential as functional food ingredients, with preclinical studies suggesting beneficial effects on gut microbial composition, inflammatory responses, oxidative stress markers, and lipid metabolism. However, the available evidence is largely derived from in vitro and animal experiments, and the efficacy of these oligosaccharides as functional-food ingredients requires further investigation. Systematic screening of marine bacterial lineages associated with seaweed tissues is a promising strategy for identifying novel CAZymes with unique substrate specificities and catalytic properties that target these diverse glucans⁶.

The genus *Exiguobacterium*, established by Collins *et al.* (1983) from alkaliphilic isolates, has subsequently been recovered from remarkably diverse habitats including creamery waste, tidal flat sediments, glacier meltwater, permafrost, deep-sea sediments, alkaline wastewater, coastal algae, and freshwater ecosystems, suggesting extraordinary metabolic versatility and adaptive capacity^{7–11}. As of July 2026, the List of Prokaryotic names with Standing in Nomenclature (LPSN) recognized 15 validly published *Exiguobacterium* species, with members spanning the phylum *Bacillota*, class *Bacilli*, and family *Exiguobacteriaceae*¹². Recent investigations have unveiled remarkable biotechnological applications of *Exiguobacterium* species across multiple sectors. In environmental remediation, select strains exhibit exceptional biosorption and bioreduction capabilities for toxic heavy metals (arsenic, cadmium, lead, chromium) and can recover rare earth elements through extracellular polysaccharides (EPS), while demonstrating biodegradative capacity for recalcitrant

compounds including dyes, petroleum hydrocarbons, and certain polymers. In sustainable agriculture, *Exiguobacterium* species function as plant growth-promoting rhizobacteria (PGPR) by producing plant hormones and enhancing stress tolerance, with commercial applications in nano-selenium biofertilizer production (US patent 12221399B2). In biotechnology and aquaculture, these bacteria produce cold-active enzymes (e.g., endoglucanases) and postbiotics with potential cosmeceutical applications, while species such as *Exiguobacterium acetylicum* serve as probiotics for aquaculture, enhancing immune responses and pathogenic resistance in finfish and crustaceans. Additionally, the distinctive orange-yellow pigments synthesized by certain *Exiguobacterium* strains exhibit potent antimicrobial activity and show promise as natural food preservatives and functional colorants^{8,13}.

In this study, we describe a novel *Exiguobacterium* strain, designated RM11^T, isolated from internal tissue of *Sargassum henslowianum*, in Quang Ngai province, Vietnam. Comprehensive characterization employing phenotypic, chemotaxonomic, and genome-based taxonomic approaches, including 16S rRNA gene phylogeny, average nucleotide identity (ANI), and digital DNA–DNA hybridization (dDDH) analyses, was conducted. The combined data support the proposal that strain RM11^T represents a novel species of the genus *Exiguobacterium*, for which the name *Exiguobacterium sargassi* sp. nov. is proposed. This discovery expands our understanding of *Exiguobacterium* species diversity and provides a platform for investigating the enzymatic mechanisms underlying algal polysaccharide degradation and exploring the potential industrial applications of this seaweed-derived strain in functional food production, environmental bioremediation, and sustainable agriculture.

2. Materials and Methods

2.1 Seaweed samples collection and culturing

Healthy *Sargassum henslowianum* samples were collected from the intertidal zone of Quang Ngai province, Vietnam, and transported to the laboratory on ice in sterile bags. To recover endophytic (tissue-associated) bacteria while excluding surface-attached epibionts, the samples were surface-sterilized and processed following established protocols for isolating endophytic bacteria from brown macroalgae with slight modification^{14,15}. Briefly, samples were rinsed three to five times in sterile seawater to remove loosely attached microorganisms, surface-sterilized by sequential immersion in 75% (v/v) ethanol for 5 min and 2.5% (w/v) sodium hypochlorite for 10 min, and rinsed seven times in sterile seawater to remove residual disinfectant. To verify that surface sterilization was effective, an aliquot of the final rinse water was spread on marine agar (MA; BD) and the sterilized surface was imprinted onto MA; only samples yielding no growth after incubation at 25–28°C for 72 h were processed further. Surface-sterilized tissue (~2 g) was macerated aseptically in sterile seawater with a sterile mortar and pestle, and the homogenate was serially diluted and spread onto a low-nutrient isolation medium composed of filtered seawater, 1% (w/v) sodium alginate, 50 mg L⁻¹ cycloheximide and 1.5% (w/v) agar to enrich for alginate-utilizing bacteria. Plates were incubated at 20°C for up to one week and observed daily. A pure culture was obtained by subculturing on MA. The isolated colonies were preserved in 20% glycerol marine broth (MB) at -80°C for long-term storage¹⁶. Then, these putative novel isolates were deposited in the Indonesian Culture Collection (InaCC) and Korean Collection for Type Cultures (KCTC).

2.2 Sequencing of 16S rRNA gene

Genomic DNA of the isolated strain was extracted using a DNA extraction kit¹⁷. The 16S rRNA genes were first amplified using the universal primers including 27F and 1492R. The complete 16S rRNA gene sequence was assembled and then searched in the EzBioCloud database to identify matching sequences¹⁸. Sequences of related strains downloaded from EzBioCloud were aligned and trimmed using BioEdit software. Subsequently, three types of phylogenetic trees were constructed utilizing the Molecular Evolutionary Genetics Analysis (MEGA X) software¹⁹: neighbor-joining (NJ)²⁰, maximum-likelihood (ML)²¹, and the maximum parsimony (MP) method²². All the phylogenetic trees were evaluated using 1,000 bootstrap iterations, with *Ectobacillus ponti* SYSU M60031^T serving as the outgroup.

2.3 Physical and biochemical analysis

Strain RM11^T was cultivated on MA to determine its characteristics. Gram reaction was performed using the BBL™ Gram Stain Kit (BD, USA). Cell morphology was examined by transmission electron microscopy (TEM) by JEM 1400 Flash (at the National Institute of Hygiene and Epidemiology, Hanoi, Vietnam). Salt tolerance was tested by preparing mimic marine broth that was modified with various concentrations of NaCl from 0% to 15% (at intervals of 1 unit) (w/v). The range and optimal temperature for growth were determined at temperature range from 4 to 50 °C on marine agar (MA). To determine the range and optimal pH, marine broth was adjusted to a pH range of 6.0–11.0 with a buffer system (at intervals of 0.5 unit), phosphate buffer for pH 6.0 to 8.0 and carbonate buffer for pH 8.5 to 11.0^{23,24}. Gliding motility was assessed by using a low nutrient soft agar²⁵. The growth under anaerobic culture condition was also

examined after 10 days of incubation at 30°C using an anaerobic jar (BD GasPak Systems) on MA supplemented with 4 mM NaNO₃ and 2 mM NaNO₂⁷. Catalase activity was conducted using 3% (v/v) hydrogen peroxide, and oxidase activity was examined with 1% (w/v) tetramethyl-p-phenylenediamine dihydrochloride, respectively. Flexirubin-type pigments were determined using a 20% (w/v) KOH solution²⁶. Additional biochemical tests were performed using API 20E (bioMérieux).

2.4 Chemotaxonomic characterization

Cells of strain RM11^T and reference strains *E. marinum* KCTC 19036^T and *E. aestuarii* KCTC 19035^T were harvested from MA after 2 days of cultivation at 30°C. The physiological stage of bacterial biomass used for fatty acid analysis was determined following the standard Sherlock Microbial Identification System method. The fatty acids of these type strains were extracted according to the MIDI protocol (Sherlock Microbial Identification System version 6.0) and then analyzed using a GC system (8890 GC system, Agilent)²⁷. The polar lipid profile was examined using the Komagata and Suzuki protocols²⁸. For polar lipid determination, compounds were spotted on a TLC plate (10×10 cm, silica gel 60 F254 plate, Merck) and then sprayed with various reagents: 0.2% ninhydrin, α-naphthol, molybdenum blue, and phosphomolybdic acid for aminolipids, glycolipids, phospholipids, and total lipids, respectively²⁸.

2.5 Genome sequencing and phylogeny

Genome sequencing of strain RM11^T was performed through a dual-platform sequencing approach that combined long-read and short-read technologies. Genomic DNA was isolated from cells cultivated on MA at 30°C for 48 h employing the QIAamp DNA Mini Kit (Qiagen, USA). DNA quality was verified by Qubit fluorometric quantification, spectrophotometric purity assessment (OD260/OD280), and 1% agarose gel electrophoresis to confirm fragment integrity. For short-read sequencing, fragment libraries were generated using the NEBNext dsDNA Fragmentase and the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, USA), and sequenced as 150 bp paired-end reads on an MGI DNBSeg-G99 platform. Long-read sequencing was conducted on the Oxford Nanopore Technologies (ONT) MinION platform. All sequencing services were provided by KTEST (Ho Chi Minh City, Vietnam). Long-read quality was evaluated with NanoComp v1.20.0²⁹, and low-quality bases and adapter sequences were trimmed using Filtlong v0.2.1 (<https://github.com/rrwick/Filtlong>). Short reads were processed with fastp v0.23.2³⁰ to remove adapters, low-quality bases (Phred score ≤ 20), ambiguous nucleotides, and duplicate reads. The filtered reads were assembled de novo using two independent pipelines: (i) Unicycler v0.4.8 in hybrid mode, and (ii) the Flye–Medaka–POLCA workflow, which performs long-read assembly with Flye, signal-level error correction with Medaka, and short-read polishing with POLCA^{31,32}; the assembly yielding higher contiguity and accuracy was retained for subsequent analyses. Assembly statistics were generated using QUAST v5.2.0, and genome completeness and contamination were assessed with CheckM v1.2.2³³ and BUSCO v5.4.4³⁴, respectively. The assembled genome was deposited in NCBI GenBank. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (dDDH) values were computed using the ANI calculator (<https://www.ezbiocloud.net/tools/ani>)³⁵ and the Genome-to-Genome Distance Calculator v3.0 (DSMZ; <https://ggdc.dsmz.de/ggdc.php>)³⁶. A phylogenetic tree based on the whole genome of strain RM11^T and closely related type strains of the genus *Exiguobacterium*, with *Ectobacillus ponti* SYSU M60031^T as the outgroup was constructed using the UBCG pipeline³⁷.

2.6 Functional genome annotation

The complete genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP)³⁸. A circular genome map was generated with the CGView server (<https://stothardresearch.ca/cgview/>). Metabolic pathways were reconstructed through the BlastKOALA server and subsequently processed with KEGG-decoder³⁹ to obtain pathway-completeness scores. Antibiotic-resistance genes were screened against the Comprehensive Antibiotic Resistance Database CARD (CARD)⁴⁰. The genomes were annotated for CAZymes using the dbCAN3 server⁴¹. The RAST annotation was additionally used to identify the metabolic pathway and functional potential of both species in the genus *Exiguobacterium*⁴².

2.7. Phenotypic characterization

The polysaccharide degradation abilities of RM11^T were determined by culturing on sole carbon sources in artificial sea water. The isolated strain was grown on solid media containing 60% (v/v) seawater, 0.1% (w/v) (NH₄)₂SO₄, and 1% (w/v) of each test polysaccharide (chitin, sodium alginate, and starch)²⁴. Polysaccharide degradation was indicated by clear zones around colonies. Furthermore, Lugol's solution was also used to visualise the hydrolysis zones (**Figure S2**).

3. Results

3.1 Novel strain isolation and morphology

During a survey of seaweed-associated bacteria from the coast of central Vietnam, strain RM11^T was recovered from the internal tissue of *Sargassum henslowianum*. On MA, the isolate produced yellow, circular, smooth colonies. TEM examination revealed that cells are ovoid to short rods, approximately 0.2–0.3 µm in width and 0.3–0.5 µm in length (based on measurements of representative cells from exponential-phase cultures), motile by means of peritrichous flagella, and occurring singly, in pairs or in small clusters (Fig. 3).

3.2 16S rRNA gene-based phylogeny

Phylogenetic analysis of the 16S rRNA gene sequence confirmed that strain RM11^T is affiliated with the genus *Exiguobacterium* within the phylum *Bacillota*. The highest sequence similarities were observed with *E. qingdaonense* S82^T (99.82%), *E. aestuarii* TF-16^T (99.74%), *E. profundum* 10C^T and *E. marinum* DSM 16307^T (99.38%). In all three tree-reconstruction algorithms (ML, NJ and MP), strain RM11^T formed a monophyletic cluster with *E. aestuarii* TF-16^T and *E. marinum* DSM 16307^T (Fig. 1), which were therefore selected as reference strains for phenotypic and chemotaxonomic comparison. The 16S rRNA gene sequence was deposited in GenBank under accession number PZ584512.

3.3 Physical and biochemical characterization

Strain RM11^T was Gram-variable, facultatively anaerobic and motile by peritrichous flagella. Gram staining yielded a variable reaction, as has been reported for other members of the genus depending on growth phase and culture conditions. Growth occurred at 10–45°C, pH 7.0–10.0 and 1–12% (w/v) NaCl. Catalase was positive and oxidase was negative. In the API 20E tests, the strain was positive for β-galactosidase (ONPG), arginine dihydrolase (ADH), citrate utilisation (CIT), acetoin production (VP) and gelatin hydrolysis (GEL), whereas it was negative for lysine decarboxylase, ornithine decarboxylase, H₂S production, urease, tryptophan deaminase, indole production and acid formation from amygdalin, arabinose, glucose, inositol, mannitol, melibiose, sorbitol, rhamnose and saccharose. A comparison of the differential physiological and biochemical properties of strain RM11^T, *E. aestuarii* TF-16^T and *E. marinum* DSM 16307^T is presented in Table 1.

Table 1
Differential physiological properties of strain RM11^T and closely related species in the genus *Exiguobacterium*. Strains: 1, RM11^T; 2, *E. aestuarii* KCTC 19035^T; 3, *E. marinum* KCTC 19036^T. All data are from this study. +, Positive; –, Negative.

characteristics	1	2	3
Habitat	Inside seaweed	Slime*	Seawater*
Flagellum type	Peritrichous	Peritrichous*	Single polar*
Oxidase	+	–	–
Range of temperature (°C)	10–45	10–47*	10–43*
Range of pH (optimal)	7.0–10.0	5.0–10.5*	5.0–10.5*
Growth at 17% NaCl	–	+	–
API 20 E			
β-galactosidase (ONPG)	+	–	–
arginine dihydrolase (ADH)	+	–	–
citrate utilization (CIT)	+	–	–
acetoin production (VP)	+	–	–
*Data collected from the article ⁸			

3.4 Chemotaxonomic characterization

The fatty acid profiles of strain RM11^T, *E. aestuarii* TF-16^T and *E. marinum* DSM 16307^T shared a similar feature. The major fatty acids of strain RM11^T were iso-C_{17:0} (16.7%), iso-C_{13:0} (13.0%) and anteiso-C_{13:0} (11.7%), followed by C_{16:0} (10.5%); the two reference strains

showed a broadly similar profile, likewise dominated by iso-C_{17:0}, anteiso-C_{13:0} and iso-C_{13:0} (Table 2). The polar lipid composition of strain RM11^T included diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), and unidentified phospholipid (PL) (**Figure S1**). The profile of polar lipid of isolated strain showed slight differences compared with the two reference strains. The two reference strains possess phosphatidylinositol and phosphatidylserine, which were absent in strain RM11^T⁸. The major polar lipids of RM11^T are consistent with the polar lipid profiles of the genus *Exiguobacterium*⁴³.

Table 2

Cellular fatty acid compositions of strain RM11^T and the closely related species. Strains: 1, RM11^T; 2, *E. aestuarii* KCTC 19035^T; 3, *E. marinum* KCTC 19036^T.

Fatty acid	1	2	3
Saturated straight-chain			
C _{12:0}	1	1.3	1.2
C _{14:0}	1.3	1.2	0.6
C_{16:0}	10.5	8.5	9.1
C _{18:0}	8.3	7.5	5.2
Saturated branched-chain			
iso-C _{12:0}	3.1	2.3	3.8
iso-C_{13:0}	13.0	10.2	12.4
anteiso-C_{13:0}	11.7	12.0	17.8
iso-C _{14:0}	2.8	1.7	1.0
anteiso-C _{14:0}	–	1.7	–
iso-C _{15:0}	8.6	6.4	6.4
anteiso-C _{15:0}	3.3	2.5	2.5
iso-C _{16:0}	5.1	3.4	4.4
iso-C_{17:0}	16.7	14.4	24.5
anteiso-C _{17:0}	3.7	3.8	5.1
iso-C _{18:0}	2.9	2.0	2.2
iso-C _{19:0}	0.6	1.5	1.0
Unsaturated			
iso-C _{17:1} ω10c	1.3	–	0.9
C _{18:1} ω9c	0.7	3.6	–
C _{18:3} ω6c (6,9,12)	–	1.0	–
Hydroxy and alcohol			
C _{16:1} 2-OH	–	0.8	–
C _{16:0} N-alcohol	0.5	0.6	–
C _{16:1} ω7c alcohol	0.6	–	–
C _{16:1} ω11c	–	0.8	0.8
Summed feature			
Summed feature 1	1.1	1.5	–
–, not detected or amounting to < 0.5%. Summed feature 1 comprised C _{15:1} iso H and/or C _{13:0} 3-OH; Summed feature 8 comprised C _{18:1} ω7c and/or C _{18:1} ω6c.			

Fatty acid	1	2	3
Saturated straight-chain			
Summed feature 8	1.7	4.9	–
–, not detected or amounting to < 0.5%. Summed feature 1 comprised C _{15:1} iso H and/or C _{13:0} 3-OH; Summed feature 8 comprised C _{18:1} ω7c and/or C _{18:1} ω6c.			

3.5 Genome analysis and taxonomic placement

The genome completeness of strain RM11^T was 99.34%, with 0.00% contamination and 0.00% strain heterogeneity, based on a lineage-specific marker-gene analysis, confirming a high-quality, near-complete genome assembly. The genome of strain RM11^T was assembled into two contigs with a total length of 2,859,837 bp and a G + C content of 47.79%: a main contig of 2,817,675 bp and a second, considerably smaller contig of 42,162 bp. The ANI values between strain RM11^T and its closest relatives *E. aestuarii* TF-16^T and *E. marinum* DSM 16307^T, *E. qingdaonense* S82^T and *E. profundum* 10C^T were 90.92%, 87.65%, 86.46% and 87.87% respectively (Table S1), and the dDDH values between strain RM11^T and its closest relatives ranged from 30.5 to 41.7%. Both the ANI and dDDH values are lower than the cutoff values of species differentiation, which are 95–96% for ANI and 70% for dDDH^{35,36}. Thus, the ANI and dDDH values confirm the novelty of strain RM11^T. A genome-based phylogenetic tree further showed monophyletic clustering of strain RM11^T with *E. aestuarii* TF-16^T and *E. marinum* DSM 16307^T, consistent with the 16S rRNA gene-based phylogenetic trees (Figs. 1 and 2).

3.6 Whole-genome functional analysis

3.6.1 Subsystem annotation (RAST)

RAST annotation of the genome of strain RM11^T identified 3,166 coding sequences (CDSs) and 46 RNAs (Fig. 4). Of these, 795 CDSs (25.1%) were assigned to 227 distinct subsystems organised into 25 broad categories; the remaining 74.9% were not assigned to any defined subsystem, consistent with the typically incomplete subsystem coverage observed for non-model organisms. The dominant categories in strain RM11^T were Amino Acids and Derivatives (193 assignments), Protein Metabolism (192), Carbohydrates (133), Cofactors, Vitamins, Prosthetic Groups and Pigments (119), and Nucleosides and Nucleotides (93).

Comparison with *E. aestuarii* TF-16^T (1,216 feature-role assignments; 837 features in 26 categories) and *E. marinum* TF-80^T (1,054 assignments; 743 features in 26 categories) revealed broadly similar functional profiles. Protein Metabolism was more strongly represented in strain RM11^T (192 assignments) than in *E. aestuarii* (155) or *E. marinum* (158), whereas Carbohydrates showed comparable representation among all three strains (133, 145 and 143 assignments, respectively). More pronounced differences were observed for Cell Division and Cell Cycle (3, 19 and 3 assignments), Cell Wall and Capsule (34, 47 and 23) and Phosphorus Metabolism (23, 29 and 2). These distributions represent predicted functional annotations and should not be interpreted as direct measurements of pathway activity.

3.6.2 Genomic features related to the metabolism of heavy metals, nitrogen, sulfur, and stress-related loci

KEGG and RAST analyses identified genes for heavy-metal transport and homeostasis in strain RM11^T, including arsenic-resistance components (arsR, arsA; arsC absent) and transporters for magnesium/cobalt/nickel (CorA), copper (CopA), ferric iron (AfuABC), and zinc (ZnuABC) (Fig. 4 and Supplementary Excel files).

For nitrogen, the genome encodes ammonia-assimilation genes (glnA, two copies; gltBD; gudB) and an incomplete/partial arginine-deiminase pathway^{44,45} but lacks classical dissimilatory nitrate-reductase or denitrification genes (narGHIJ, napAB, nirBKS, norBC, nosZ); RAST subsystem labels for nitrogen metabolism were not corroborated by these KEGG marker genes, so no denitrification or dissimilatory nitrogen-cycling capacity is claimed for strain RM11^T.

RAST and Prokka annotations identified a broad stress-response gene set in strain RM11^T (35 RAST features across 13 subsystems), dominated by choline/betaine osmoprotection genes (8 features) and oxidative-stress genes (7 features), including glutathione redox-cycle components⁴⁶, superoxide dismutase (sodA, Mn-dependent) and catalase (katE, two copies), together with cell-envelope-stress genes, osmotic-stress genes (glycine-betaine biosynthesis/transport betA, opuBA/opuBB; mechanosensitive channels mscL, mscS),

thioredoxin genes (trxA, trxB), dimethylarginine-metabolism genes, and molecular chaperones/heat-shock proteins (dnaK, groEL/groES, htpG, clpB, cspA)^{47,48}. This repertoire is consistent with adaptation to fluctuating oxidative, osmotic and thermal conditions typical of the marine and seaweed-associated environment, although such genes are widespread among bacteria and do not by themselves establish niche-specific adaptation²⁴.

3.6.3 Comparative KEGG-Decoder functional analysis

To place strain RM11^T within the metabolic context of the genus, KEGG-decoder pathway-completeness scores were compared across RM11^T and six reference type strains (Fig. 5). KEGG-decoder scores quantify the completeness of KEGG Orthology (KO) assignments for each category; a score of zero indicates that none of the marker KOs required for that category was detected under the applied annotation pipeline and does not demonstrate biological absence of every gene potentially contributing to that function³⁹.

Accordingly, the following observations are framed as genomic potential. Of the 169 categories assessed, 109 scored zero in all seven strains, 28 scored 1.00 in all strains, one scored 0.99 in all strains, 10 were invariant at intermediate completeness scores, and 21 varied among strains (Supplementary Excel file). No category was uniquely present or uniquely absent in RM11^T relative to all six reference strains, indicating that RM11^T conforms closely to the conserved metabolic framework of *Exiguobacterium*^{49,50}.

Central carbon and carbohydrate metabolism were conserved across all strains. Glycolysis (RM11^T score = 0.89) and a partial TCA cycle (0.38) fell within the reference range. Starch/glycogen degradation was complete, whereas the synthesis module was consistently near-complete (0.99) across all seven genomes, and α -amylase, pullulanase and β -glucosidase each scored 1.00 in every strain. This mirrors the genus-wide conservation of the GH13 and GH57 starch-degrading families reported by Tian et al.⁵⁰, in which the GH13_5, GH13_14, GH13_29 and GH13_31 subfamilies were present in all 187 genomes surveyed. The α -glucan-metabolising capacity of RM11^T is therefore a conserved genus-level trait rather than a species-specific attribute. The strain-level dbCAN3 analysis (Table 3) adds resolution that pathway mapping does not capture, GH13 subfamily composition, domain architecture, and the predicted secreted GH16_3–CBM4 β -glucanase candidate and the two approaches are treated here as complementary evidence layers: KEGG-decoder for pathway completeness and dbCAN3 for family/subfamily and domain-level detail.

Table 3
Distribution of carbohydrate-active enzyme (CAZyme) classes in the genome of strain RM11^T (*Exiguobacterium sargassi* sp. nov.). AA: Auxiliary activities, CBM: carbohydrate-binding modules, CE: carbohydrate esterase, GH: Glycoside hydrolase, GT: Glycosyl transferase.

CAZyme type	number
AA	1
CBM	10
CE	7
GH	39
GT	26
PL	0
Total CAZymes	74

Canonical cellulase, glucoamylase, pectinesterase and chitinase functions were not recovered by KEGG-decoder in RM11^T or any reference strain. This should be interpreted cautiously, because β -glucosidase (GH1/GH3) and β -N-acetylhexosaminidase markers do not by themselves establish depolymerisation of cellulose or chitin polymers. The data support downstream utilisation of soluble β -glucosides and N-acetylhexosamine-containing oligomers in RM11^T but do not establish extracellular depolymerisation of structural biopolymers.

All dissimilatory nitrogen-cycling categories (dissimilatory nitrate reduction, DNRA, nitrite, nitric-oxide and nitrous-oxide reduction, and nitrogen fixation) scored zero in RM11^T and every reference strain. This shared absence among the seven genomes examined is consistent with the analysis of 18 complete *Exiguobacterium* genomes reported by Tian et al.⁵⁰. The *glnA*, *gltBD* and *gudB* genes noted therefore function in assimilatory ammonium incorporation via the GS–GOGAT cycle, and no dissimilatory nitrogen cycling should be inferred. Dissimilatory sulfate reduction and the Sox system were likewise not recovered in any strain. One sulfur category, predicted sulfide oxidation, scored 1.00 in RM11^T, *E. aestuarii*, *E. marinum* and *E. algae* but zero in *E. mexicanum*, *E. profundum* and *E. qingdaonense*. If this score corresponds to sulfide:quinone oxidoreductase, it would represent a detoxification rather than a lithotrophic function⁵¹; it is a feature shared by four of the seven strains examined, and verification of the underlying KO and candidate *sqr* gene is required before any functional or ecological role is assigned.

Among the seven genomes examined, RM11^T displayed a distinctive combination of an undetected ferrous-iron transporter FeoB marker (score = 0.00) and a detected NRAMP-family Fe/Mn transporter MntH (1.00). FeoB was scored present in *E. profundum*, *E. algae*, *E. aestuarii* and *E. marinum*, and MntH in *E. aestuarii*, *E. marinum* and *E. algae*; an AfuA-like ferric-iron substrate-binding component scored 1.00 in all strains. In prokaryotes, the Feo system is the principal dedicated Fe(II) uptake route and is typically favoured under reducing conditions, whereas MntH preferentially transports Mn(II) but can also accept Fe(II)^{52,53}. Because this pattern was inferred from KEGG-based annotation of single representatives, it should not be regarded as a species-level trait: the canonical FeoB marker was not detected in RM11^T, but alternative Fe(II)-uptake routes, including MntH-mediated transport, cannot be excluded, and confirmation requires gene-level searching for *feoB* (K04759), together with inspection of operon structure and synteny. This transporter configuration may be compatible with metal acquisition under oxic or oxidative-stress conditions, but its relationship to the physicochemical environment within *Sargassum* tissue remains hypothetical and is not developed further here.

The remaining variable categories reflected genomic potential broadly shared across the genus. A metal-homeostasis-associated transporter profile was detected in RM11^T: the copper-transporting ATPase CopA, the CorA Mg²⁺ transporter (permeable to Co²⁺/Ni²⁺ but not a direct cobalt-resistance determinant), and a partial arsenic-resistance-associated module. The absence of a detectable *arsC* gene does not support a complete arsenate-reduction pathway, although arsenite efflux or other arsenic-homeostasis mechanisms remain possible; this parallels Tian et al. (2026)⁵⁰, who reported *arsB* in 187/187 but *arsC* in only 15/187 genomes. Riboflavin biosynthesis was complete (1.00) in RM11^T, whereas the low cobalamin-biosynthesis score (0.12) does not support a complete canonical de novo cobalamin-biosynthesis pathway; uptake, salvage and alternative routes were not assessed. Complete KEGG-decoder modules for threonine, methionine, phenylalanine and tyrosine biosynthesis were not recovered in any strain and are reported as unrecovered modules rather than confirmed auxotrophies, which would require growth testing in defined medium. Asparagine biosynthesis scored zero in RM11^T yet Tian et al. (2026) reported asparagine synthesis in all 18 complete genomes; this discrepancy warrants direct inspection of *asnA/asnB* and alternative routes rather than a conclusion of asparagine auxotrophy.

Flagellar biosynthesis (0.83) and chemotaxis (0.75) were conserved, consistent with the peritrichous flagella observed by transmission electron microscopy and with the conservation of flagellar and chemotaxis operons across the genus. The Sec-SRP pathway was near-complete (0.81) and a partial twin-arginine translocation (Tat) module (0.50) was detected; the predicted GH16_3–CBM4 protein carried a signal peptide consistent with extracellular targeting, but whether it is exported through the Sec or Tat route requires signal-peptide-class-specific analysis, as the Tat pathway handles only proteins bearing a twin-arginine motif. No complete specialised secretion-system (Type I–VI) modules were identified by KEGG-decoder in the seven genomes. Overall, the KEGG-decoder comparison places RM11^T firmly within the conserved metabolic architecture of *Exiguobacterium*, with no category uniquely present or absent relative to the reference strains. The contribution to species novelty therefore rests on the polyphasic taxonomic evidence and on the finer-grained CAZyme diversity resolved by dbCAN3, while the transporter-level differences noted above define targets for gene-level and experimental follow-up rather than established adaptive traits.

3.6.4 Antimicrobial resistance screening

No acquired antimicrobial resistance genes were identified when the genome was screened against the Comprehensive Antibiotic Resistance Database (CARD), AMRFinderPlus or ResFinder-type reference sequences. This finding suggests that strain RM11^T does not carry horizontally acquired resistance determinants, consistent with its non-clinical origin.

3.6.5 Phenotypic characterization

The isolated strain RM11^T was grown on Petri dishes containing sole carbon sources. Therefore, the experiments demonstrated the polysaccharide-degradation abilities of the strain.

3.7 Annotation of carbohydrate-active enzymes (CAZymes) through dbCAN meta server

The dbCAN3 annotation identified 138 candidate CAZyme proteins, of which 74 were supported by at least two of the three tools (HMMER, DIAMOND, dbCAN-sub) and were retained as the reliable dataset for further analysis⁵⁴. Among these, 39 proteins carried glycoside hydrolase (GH) domains, 26 carried glycosyltransferase (GT) domains, 10 possessed carbohydrate-binding modules (CBMs), seven were carbohydrate esterases (CEs), one was an auxiliary activity (AA) protein, and no reliable polysaccharide lyase (PL) was detected (Table 3)⁵⁵. Nine proteins had multi-domain architectures spanning more than one class, so class counts sum to more than 74. GH was the dominant class, followed by GT, indicating a repertoire biased towards carbohydrate hydrolysis rather than glycan biosynthesis alone.

The most prominent feature was a relatively abundant repertoire of GH13 family members (14 proteins across seven subfamilies: GH13_5, _14, _20, _29, _31, _41, _45), several coupled to starch-binding CBM34/CBM41/CBM48 modules, consistent with activity on diverse α -glucans including starch, amylose, amylopectin, glycogen, maltodextrin and pullulan. Two proteins, (GH13_14–CBM41/CBM48) and (GH13_41), carry signal peptides suggestive of extracellular α -glucan degradation, although subfamily-level annotation alone cannot assign precise substrate specificity and requires experimental confirmation⁵⁶. GH57, GH65, GT5 and GT35 further indicate that α -glucan metabolism may operate bidirectionally, supporting both exogenous starch utilisation and intracellular glycogen synthesis/mobilisation.

A single secreted GH16_3 CBM4 protein, supported by all three tools, was predicted to act on β -1,3- or β -1,3/1,4-glucans (e.g. laminarin, lichenan), representing the strain's principal β -glucan-degrading candidate; because GH16 is multispecific, its precise substrate requires individual testing⁵⁷. Additional GH families (GH32, GH36, GH42, GH1, GH3) suggest activity on assorted disaccharides and oligosaccharides. Two independent loci were consistently assigned by both HMMER and dbCAN-sub to sulfoquinovoside/hexosaminidase-related families: GH188 (EC 3.2.1.199), compatible with utilisation of sulfolipid-derived sugars from photosynthetic membranes, and GH179 (EC 3.2.1.52), more consistent with β -N-acetylhexosaminidase activity. A third, separate locus, contig_1_1474 (EC 3.2.1.52), was discordantly assigned by HMMER (GH188) and dbCAN-sub (GH179); this single locus, not contig_1_2519, is the unresolved case and requires further motif- or structure-level analysis before a family can be assigned with confidence.

A further subset of genes (GT51, GH73, GH23, GH171, CE4–CBM50) is more plausibly linked to peptidoglycan synthesis, remodelling and recycling, potentially serving cell division, autolysis or biofilm formation, than to environmental polysaccharide degradation⁵⁸. GT was the second most abundant class overall (chiefly GT4 and GT2), consistent with roles in exopolysaccharide, membrane-glycan or peptidoglycan biosynthesis; because GTs form rather than break glycosidic bonds, degradation potential should be assessed from the GH/CE/PL/CBM composition and signal-peptide status rather than from total CAZyme counts.

Seventeen proteins carried predicted signal peptides (14 with ≥ 2 -tool support, $\sim 18.9\%$ of the reliable dataset), including the principal GH13 and GH16_3 candidates, consistent with a two-stage strategy of extracellular polysaccharide breakdown followed by intracellular metabolism of the resulting oligo- and monosaccharides (noting that SignalP-negative proteins may still be secreted via non-classical routes). Consistent with this, the genome showed strong predicted capacity for starch/ α -glucan utilisation and a candidate β -glucan-degrading enzyme, but lacked hallmark cellulase (GH6, GH7, GH48) and xylanase (GH10, GH11) genes and any reliable polysaccharide lyase, and its single AA1 protein (no signal peptide) does not support a laccase or lignin-degrading function. Overall, the CAZyme profile is consistent with an organism specialised in α -glucan, β -glucan and oligosaccharide utilisation rather than a broad-spectrum degrader of cellulose, hemicellulose, pectin or lignin.

For context, Muhammad et al.²⁴ reported 57 CAZymes in *Pontimicrobium maritimus* SW4^T and 55 in *P. aquaticum*, both GT-dominant; strain RM11^T instead shows a GH-biased profile (GH/GT ≈ 1.5), suggesting comparatively stronger predicted carbohydrate hydrolytic potential, although cross-study comparisons of raw counts are sensitive to genome size, database version and multi-domain handling. This α -glucan/ β -glucan-oriented, cell-wall-active repertoire, lacking recognisable alginate- or cellulose-degrading systems, is compatible with a tissue-associated lifestyle in which RM11^T accesses host-derived laminarin and sulfolipid-derived sugars rather than structural algal polysaccharides; the identity of the environmental α -glucan substrate(s) supporting the GH13 expansion remains uncertain and is not assumed to be a normal *Sargassum* storage polysaccharide¹⁴. Priority targets for functional validation include the GH13 starch-active proteins, the GH16_3–CBM4 β -glucanase candidate, and the peptidoglycan-active proteins, via enzyme assays on defined substrates, product analysis by chromatography/mass spectrometry, and expression profiling under varying carbon sources.

Discussion

The polyphasic evidence unequivocally supports the classification of strain RM11^T as a novel species within the genus *Exiguobacterium*. The 16S rRNA gene similarity to *E. qingdaonense* S82^T (99.82%) exceeds the threshold commonly associated with species-level distinctness, yet both ANI (86.46%) and dDDH (30.5%) values fall far below the accepted species-delineation cut-offs (95–96% and 70%, respectively)^{35,36}, demonstrating that high 16S rRNA similarity alone does not predict genomic coherence within this genus. The congruence between 16S rRNA gene-based and UBCG genome-based phylogenies, together with differential phenotypic and chemotaxonomic characteristics (Table 1), reinforces the proposal of *E. sargassi* sp. nov.

A notable observation concerns the isolation conditions. Strain RM11^T was recovered on a medium containing sodium alginate as the sole supplementary carbon source, yet its genome encodes no detectable polysaccharide lyase (PL) genes. Several, non-mutually-exclusive, explanations may account for this apparent paradox: (i) the strain may have utilised trace nutrients present in the filtered seawater base of the isolation medium rather than alginate itself; (ii) alginate oligosaccharides released by co-occurring alginate-degrading organisms during the initial enrichment step may have sustained growth through cross-feeding; or (iii) the strain may degrade or modify alginate through a mechanism not captured by current PL family definitions in the CAZy database. Regardless of the explanation, the absence of PL genes underscores that isolation on a particular substrate does not guarantee that the isolate is capable of its enzymatic degradation, a consideration that is relevant to any enrichment-based screening strategy.

The KEGG-decoder comparison revealed that strain RM11^T conforms closely to the conserved metabolic framework of the genus *Exiguobacterium*, with no pathway category uniquely present or absent relative to six reference type strains. Among the iron-transport markers, RM11^T displayed a distinctive combination of an undetected FeoB ferrous-iron transporter and a detected NRAMP-family MntH transporter. In prokaryotes, the Feo system is the principal dedicated Fe(II) uptake route, whereas MntH preferentially transports Mn(II) but can also accept Fe(II)^{52,53}. Because this pattern was inferred from single representatives, it should not be regarded as a species-level trait; confirmation requires gene-level analysis of *feoB* (K04759) together with inspection of operon structure and synteny. The remaining variable categories-CopA, CorA Mg²⁺ transporter, partial arsenic-resistance module (*arsC* absent, paralleling Tian *et al.*⁵⁰) reflect genomic potential broadly shared across the genus.

The CAZyme repertoire of strain RM11^T is dominated by glycoside hydrolases rather than glycosyltransferases (GH/GT $\approx$ 1.5), in contrast to the GT-dominant profiles reported for *Pontimicrobium maritimus* SW4^T and *P. aquaticum* (57 and 55 CAZymes, respectively)²⁴, although cross-study comparisons of raw counts are sensitive to genome size, database version and multi-domain handling. Within the GH complement, the 14 GH13-family proteins across seven subfamilies represent the genus-wide conservation of α -glucan-metabolising capacity documented by Tian *et al.*⁵⁰ rather than a species-specific expansion. Nevertheless, the strain-level subfamily composition (GH13_5, _14, _20, _29, _31, _41, _45), domain architectures and the predicted secreted GH16_3–CBM4 β -glucanase provide resolution that pathway-level mapping does not capture. This α -glucan/ β -glucan-oriented, cell-wall-active repertoire, lacking recognisable alginate- or cellulose-degrading systems, is compatible with a tissue-associated lifestyle in which RM11^T accesses host-derived laminarin and sulfolipid-derived sugars rather than structural algal polysaccharides¹⁴. Priority targets for functional validation include the GH13 starch-active proteins, the GH16_3–CBM4 β -glucanase candidate and the peptidoglycan-remodelling proteins.

Conclusion

Strain RM11^T, isolated from the internal tissue of the brown seaweed *Sargassum henslowianum*, represents a novel species within the genus *Exiguobacterium* (family *Exiguobacteriaceae*, phylum *Bacillota*). This conclusion is supported by 16S rRNA gene phylogeny, ANI and dDDH values that fall below the accepted species-delineation thresholds, consistent phenotypic and chemotaxonomic differentiation from the closest relatives, and a genome encoding a GH-dominated CAZyme repertoire enriched in α -glucan-active GH13 family members with a candidate secreted GH16_3–CBM4 β -glucanase but no polysaccharide lyase genes. We therefore propose the name *Exiguobacterium sargassi* sp. nov. for this taxon, with strain RM11^T (= KCTC 43930^T = InaCC B1791^T) as the type strain.

Description of *Exiguobacterium sargassi* sp. nov.

Exiguobacterium sargassi (*sar.gas'si*. N.L. gen. n. *sargassi*, of *Sargassum*, a genus of brown seaweed, reflecting the isolation source, the internal tissue of *Sargassum henslowianum*).

Cells are Gram-variable, facultatively anaerobic and motile by means of peritrichous flagella. Colonies on MA are light yellow, circular and smooth. Growth occurs at 10–45°C, pH 7.0–10.0 and 1–12% (w/v) NaCl. In the API 20E system, the strain tests positive for β -galactosidase (ONPG), arginine dihydrolase (ADH), citrate utilisation (CIT), acetoin production (VP) and gelatin hydrolysis (GEL); negative for lysine decarboxylase, ornithine decarboxylase, H₂S production, urease, tryptophan deaminase, indole production, oxidase and acid formation from mannitol, inositol, sorbitol, rhamnose, saccharose, melibiose, amygdalin, arabinose and glucose. The major fatty acids are iso-C_{17:0}, iso-C_{13:0} and anteiso-C_{13:0}. The polar lipids comprise phosphatidylethanolamine (PE), diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and unidentified polar lipids. The genomic DNA G + C content of the type strain is 47.79%.

The type strain, RM11^T (= KCTC 43930^T = InaCC B1791^T), was isolated from the internal tissue of the brown seaweed *Sargassum henslowianum* collected in Quang Ngai province, Vietnam. The 16S rRNA gene sequence was deposited in NCBI GenBank under accession number PZ584512. The whole-genome sequence was deposited under accession number JCBAOU000000000.1.

Declarations

Conflicts of interest

The authors declare that there are no conflicts of interest.

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Author Contribution

Ho Le Han performed experiments of isolation, identification, and characterization of the bacterial strains including polar lipids, fatty acid analysis, genome analysis for RAST subsystem. H.L.H. also wrote the manuscript. Tien Quang Vuong performed genomic and KEGG analyses, constructed a UBGC genome tree. Thi Dong Phuong Nguyen analysed genomes and contributed to manuscript writing. Song-Gun Kim supervised the experiments and contributed to manuscript writing. Minh Nhat Dang supervised the experiments and finalized the manuscript. All authors reviewed the manuscript.

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Data Availability

Data availability statement The datasets generated and/or analysed during the current study are available with the GenBank accession number PZ584512 and JCBAOU000000000.1 <https://www.ncbi.nlm.nih.gov/nuccore/PZ584512.1> <https://www.ncbi.nlm.nih.gov/nuccore/JCBAOU000000000.1/>.

References

1. Mabeau, S. & Kloareg, B. Isolation and Analysis of the Cell Walls of Brown Algae: *Fucus spiralis*, *F. ceranoides*, *F. vesiculosus*, *F. serratus*, *Bifurcaria bifurcata* and *Laminaria digitata*. *Journal of experimental botany* 38, 1573–1580, (1987). 10.1093/jxb/38.9.1573%J *Journal of Experimental Botany*.
2. Donati, I. & Paoletti, S. in *in Alginates: Biology and Applications*. 1–53 (eds Rehm, B. H. A.) (Springer, 2009).
3. Ertesvåg, H. Alginate-modifying enzymes: biological roles and biotechnological uses. *Front. Microbiol.* Volume 6– (2015). 10.3389/fmicb.2015.00523 (2015).

4. Tanna, B. & Mishra, A. Nutraceutical Potential of Seaweed Polysaccharides: Structure, Bioactivity, Safety, and Toxicity. *Compr. Rev. Food Sci. Food Saf.* **18**, 817–831. 10.1111/1541-4337.12441 (2019).
5. Liu, M., Liu, L., Zhang, H., Yi, B. & Everaert, N. Alginate oligosaccharides preparation, biological activities and their application in livestock and poultry. *J. Integr. Agric.* **20**, 24–34. [https://doi.org/10.1016/S2095-3119\(20\)63195-1](https://doi.org/10.1016/S2095-3119(20)63195-1) (2021).
6. Kim, H. T. et al. Depolymerization of alginate into a monomeric sugar acid using Alg17C, an exo-oligoalginate lyase cloned from *Saccharophagus degradans* 2–40. *Appl. Microbiol. Biotechnol.* **93**, 2233–2239. 10.1007/s00253-012-3882-x (2012).
7. Liu, F. et al. *Exiguobacterium algae* sp. nov. and *Exiguobacterium qingdaonense* sp. nov., two novel moderately halotolerant bacteria isolated from the coastal algae. *Antonie van Leeuwenhoek*. **114**, 1399–1406. 10.1007/s10482-021-01594-8 (2021).
8. Kim, I. G. et al. *Exiguobacterium aestuarii* sp. nov. and *Exiguobacterium marinum* sp. nov., isolated from a tidal flat of the Yellow Sea in Korea. *Int J Syst Evol Microbiol.* **55**, 885–889, (2005). <https://doi.org/10.1099/ijs.0.63308-0>
9. Chaturvedi, P. & Shivaji, S. *Exiguobacterium indicum* sp. nov., a psychrophilic bacterium from the Hamta glacier of the Himalayan mountain ranges of India. *Int. J. Syst. Evol. Microbiol.* **56**, 2765–2770. <https://doi.org/10.1099/ijs.0.64508-0> (2006).
10. Rodrigues, D. F. et al. Characterization of *Exiguobacterium* isolates from the Siberian permafrost. Description of *Exiguobacterium sibiricum* sp. nov. *Extremophiles* **10**, 285–294. 10.1007/s00792-005-0497-5 (2006).
11. Dastager, S. G. et al. *Exiguobacterium enclense* sp. nov., isolated from sediment. *Int. J. Syst. Evol. Microbiol.* **65**, 1611–1616. 10.1099/ijs.0.000149 (2015).
12. Freese, H. M., Meier-Kolthoff, J. P., Sardà Carbasse, J., Afolayan, Ayorinde, O. & Göker, M. TYGS and LPSN in 2025: a Global Core Biodata Resource for genome-based classification and nomenclature of prokaryotes within DSMZ Digital Diversity. *Nucleic acids research* **54**, D884–D891, (2026). 10.1093/nar/gkaf1110%J Nucleic Acids Research.
13. Frühling, A., Schumann, P., Hippe, H., Sträubler, B. & Stackebrandt, E. *Exiguobacterium undae* sp. nov. and *Exiguobacterium antarcticum* sp. nov. *Int. J. Syst. Evol. Microbiol.* **52**, 1171–1176. 10.1099/00207713-52-4-1171 (2002).
14. Li, Y. et al. Community structure of endophytic bacteria of *Sargassum thubergii* in the intertidal zone of Qingdao in China. *AMB Express*. **14**, 35. 10.1186/s13568-024-01688-2 (2024).
15. Sahu, P. K. et al. Surface sterilization for isolation of endophytes: Ensuring what (not) to grow. *J. Basic Microbiol.* **62**, 647–668. 10.1002/jobm.202100462 (2022).
16. Le Han, H., Nguyen, T. T. H., Li, Z., Shin, N. R. & Kim, S. G. *Cellulosimicrobium protaetiae* sp. nov., isolated from the gut of the larva of *Protaetia brevitarsis seulensis*. *Int. J. Syst. Evol. Microbiol.* **72** 10.1099/ijsem.0.005296 (2022).
17. Muhammad, N., Avila, F., Nedashkovskaya, O. I. & Kim, S. G. Three novel marine species of the genus *Reichenbachiella* exhibiting degradation of complex polysaccharides. *Front. Microbiol.* **14**, 1265676 (2023).
18. Yoon, S. H. et al. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int. J. Syst. Evol. Microbiol.* **67**, 1613–1617. <https://doi.org/10.1099/ijsem.0.001755> (2017).
19. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **35**, 1547–1549. 10.1093/molbev/msy096 (2018).
20. Saitou, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425. 10.1093/oxfordjournals.molbev.a040454 (1987).
21. Felsenstein, J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376. <https://doi.org/10.1007/bf01734359> (1981).
22. Fitch, W. M. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Biology*. **20**, 406–416 (1971).
23. Muhammad, N. et al. *Flavobacterium litorale* sp. nov., isolated from red alga. *Int. J. Syst. Evol. Microbiol.* **72** <https://doi.org/10.1099/ijsem.0.005458> (2022).
24. Muhammad, N., Vuong, T. Q., Han, H. L. & Kim, S. G. Comparative genome analyses reveal insights into the marine adaptation of a novel bacterium *Pontimicrobium maritimus* sp. nov., isolated from seawater. *Sci. Rep.* **15**, 24027. 10.1038/s41598-025-07372-0 (2025).
25. Tittsler, R. P. & Sandholzer, L. A. The Use of Semi-solid Agar for the Detection of Bacterial Motility. *J. Bacteriol.* **31**, 575–580. <https://doi.org/10.1128/jb.31.6.575-580.1936> (1936).
26. Vila, E., Hornero-Méndez, D., Azziz, G., Lareo, C. & Saravia, V. Carotenoids from heterotrophic bacteria isolated from Fildes Peninsula, King George Island, Antarctica. *Biotechnol. Rep. (Amst)*. **21**, e00306. <https://doi.org/10.1016/j.btre.2019.e00306> (2019).
27. Sasser, M. C. Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids Technical Note # 101. (1990).

28. Komagata, K. & Suzuki, K. 4 Lipid and Cell-Wall Analysis in Bacterial Systematics. *Methods Microbiol.* **19**, 161–207 (1988).
29. De Coster, W., D'Hert, S., Schultz, D. T., Cruts, M. & Van Broeckhoven, C. NanoPack: visualizing and processing long-read sequencing data. *Bioinf. (Oxford England)*. **34**, 2666–2669. 10.1093/bioinformatics/bty149 (2018).
30. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinf. (Oxford England)*. **34**, i884–i890. 10.1093/bioinformatics/bty560 (2018).
31. Zimin, A. V. & Salzberg, S. L. The genome polishing tool POLCA makes fast and accurate corrections in genome assemblies. *PLoS Comput. Biol.* **16**, e1007981. 10.1371/journal.pcbi.1007981 (2020).
32. Kolmogorov, M., Yuan, J., Lin, Y. & Pevzner, P. A. Assembly of long, error-prone reads using repeat graphs. *Nat. Biotechnol.* **37**, 540–546. 10.1038/s41587-019-0072-8 (2019).
33. Parks, D. H., Imelfort, M., Skennerton, C. T., Hugenholtz, P. & Tyson, G. W. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* **25**, 1043–1055. 10.1101/gr.186072.114 (2015).
34. Tegenfeldt, F. et al. OrthoDB and BUSCO update: annotation of orthologs with wider sampling of genomes. *Nucleic acids research* **53**, D516–D522, (2025). 10.1093/nar/gkaf987%J Nucleic Acids Research.
35. Yoon, S. H., Ha, S. M., Lim, J., Kwon, S. & Chun, J. A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie Van Leeuwenhoek*. **110**, 1281–1286. <https://doi.org/10.1007/s10482-017-0844-4> (2017).
36. Meier-Kolthoff, J. P., Auch, A. F., Klenk, H. P. & Goker, M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinform.* **14**, 60. 10.1186/1471-2105-14-60 (2013).
37. Na, S. I. et al. Up-to-date bacterial core gene set and pipeline for phylogenomic tree reconstruction. *J. Microbiol.* **56**, 280–285. 10.1007/s12275-018-8014-6 (2018).
38. Li, W. et al. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. *Nucleic Acids Res.* **49**, D1020–d1028. 10.1093/nar/gkaa1105 (2021).
39. Graham, E. D., Heidelberg, J. F. & Tully, B. J. Potential for primary productivity in a globally-distributed bacterial phototroph. *Isme j.* **12**, 1861–1866. <https://doi.org/10.1038/s41396-018-0091-3> (2018).
40. McArthur, A. G. et al. The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother.* **57**, 3348–3357. 10.1128/aac.00419-13 (2013).
41. Ausland, C. et al. dbCAN-PUL: a database of experimentally characterized CAZyme gene clusters and their substrates. *Nucleic Acids Res.* **49**, D523–d528. 10.1093/nar/gkaa742 (2021).
42. Aziz, R. K. et al. The RAST Server: rapid annotations using subsystems technology. *BMC Genom.* **9** 10.1186/1471-2164-9-75 (2008).
43. Collins, M. D., Lund, B. M., Farrow, J. A. E. & Schleifer, K. H. Chemotaxonomic Study of an Alkalophilic Bacterium, *Exiguobacterium aurantiacum* gen. nov., sp. nov. *Int. J. Syst. Evol. Microbiol.* **129**, 2037–2042. <https://doi.org/10.1099/00221287-129-7-2037> (1983).
44. Liu, Y. et al. Spatial and temporal conversion of nitrogen using *Arthrobacter* sp. 24S4-2, a strain obtained from Antarctica. *Front. Microbiol.* **14**, 1040201. 10.3389/fmicb.2023.1040201 (2023).
45. Schreiber, F., Wunderlin, P., Udert, K. M. & Wells, G. F. Nitric oxide and nitrous oxide turnover in natural and engineered microbial communities: biological pathways, chemical reactions, and novel technologies. *Front. Microbiol.* **3**, 372. 10.3389/fmicb.2012.00372 (2012).
46. Kwon, D. H. et al. Protective Effect of Glutathione against Oxidative Stress-induced Cytotoxicity in RAW 264.7 Macrophages through Activating the Nuclear Factor Erythroid 2-Related Factor-2/Heme Oxygenase-1 Pathway. *Antioxid. (Basel Switzerland)*. **8** 10.3390/antiox8040082 (2019).
47. Klein, G., Laskowska, E., Taylor, A. & Lipińska, B. IbpA/B small heat-shock protein of marine bacterium *Vibrio harveyi* binds to proteins aggregated in a cell during heat shock. *Marine biotechnology (New York, N.Y.)* **3**, 346–354, (2001). 10.1007/s10126001-0009-2
48. Muchaamba, F., Wambui, J., Stephan, R. & Tasara, T. Cold Shock Proteins Promote Nisin Tolerance in *Listeria monocytogenes* Through Modulation of Cell Envelope Modification Responses. *Front. Microbiol.* **12**, 811939. 10.3389/fmicb.2021.811939 (2021).
49. Zhang, D. et al. Comparative Genomics of *Exiguobacterium* Reveals What Makes a Cosmopolitan Bacterium. *mSystems* **6**, e0038321, (2021). 10.1128/mSystems.00383-21
50. Tian, Y., Zou, L. & Ji, Y. Comprehensive genomic analyses revealed the adaptation strategies of *Exiguobacterium* and its phage genomic diversity. *Front. Microbiol.* **17**, 1841508. 10.3389/fmicb.2026.1841508 (2026).
51. Marcia, M., Ermler, U., Peng, G. & Michel, H. A new structure-based classification of sulfide:quinone oxidoreductases. *Proteins* **78**, 1073–1083. 10.1002/prot.22665 (2010).

52. Kehres, D. G. & Maguire, M. E. Emerging themes in manganese transport, biochemistry and pathogenesis in bacteria. *FEMS Microbiol. Rev.* **27**, 263–290. 10.1016/s0168-6445(03)00052-4 (2003).
53. Lau, C. K., Krewulak, K. D. & Vogel, H. J. Bacterial ferrous iron transport: the Feo system. *FEMS Microbiol. Rev.* **40**, 273–298. 10.1093/femsre/fuv049 (2016).
54. Zheng, J. et al. dbCAN3: automated carbohydrate-active enzyme and substrate annotation. *Nucleic Acids Res.* **51**, W115–w121. 10.1093/nar/gkad328 (2023).
55. Drula, E. et al. The carbohydrate-active enzyme database: functions and literature. *Nucleic Acids Res.* **50**, D571–d577. 10.1093/nar/gkab1045 (2022).
56. Janeček, Š., Svensson, B. & MacGregor, E. A. α -Amylase: an enzyme specificity found in various families of glycoside hydrolases. *Cell. Mol. Life Sci.* **71**, 1149–1170. 10.1007/s00018-013-1388-z (2014).
57. Liberato, M. V. et al. Insights into the dual cleavage activity of the GH16 laminarinase enzyme class on β -1,3 and β -1,4 glycosidic bonds. *J. Biol. Chem.* **296**, 100385. 10.1016/j.jbc.2021.100385 (2021).
58. Aragunde, H., Biarnés, X. & Planas, A. Substrate Recognition and Specificity of Chitin Deacetylases and Related Family 4 Carbohydrate Esterases. *Int. J. Mol. Sci.* **19** 10.3390/ijms19020412 (2018).

Figures

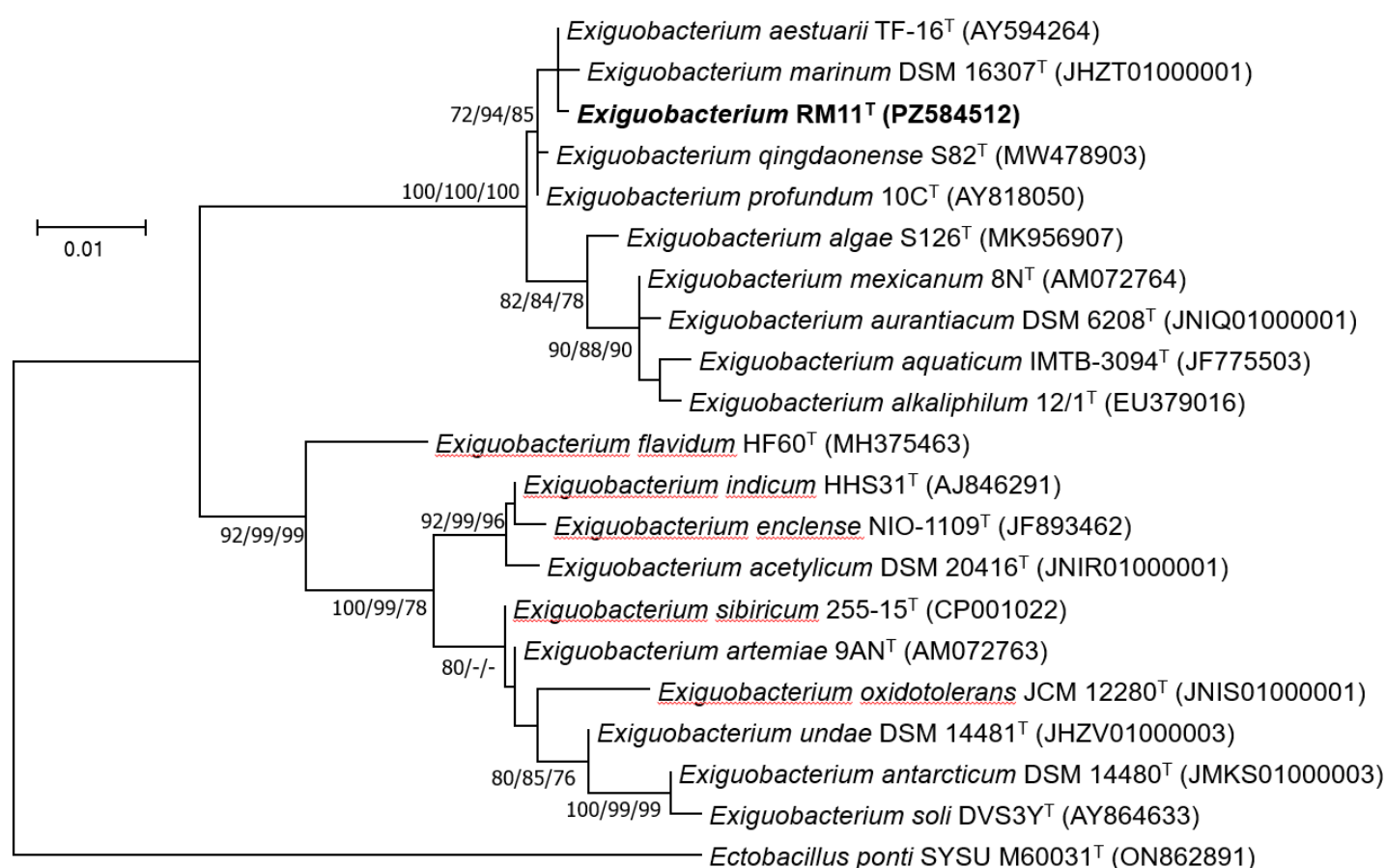


Figure 1

Maximum likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationship of strain RM11^T and closely related species in the genus *Exiguobacterium*. Bootstrap values (>70%) in the order of ML/NJ/MP are shown at the branch points based on 1000 replications. *Ectobacillus ponti* SYSU M60031^T was used as the outgroup. Bar 0.01 substitutions per nucleotide position.

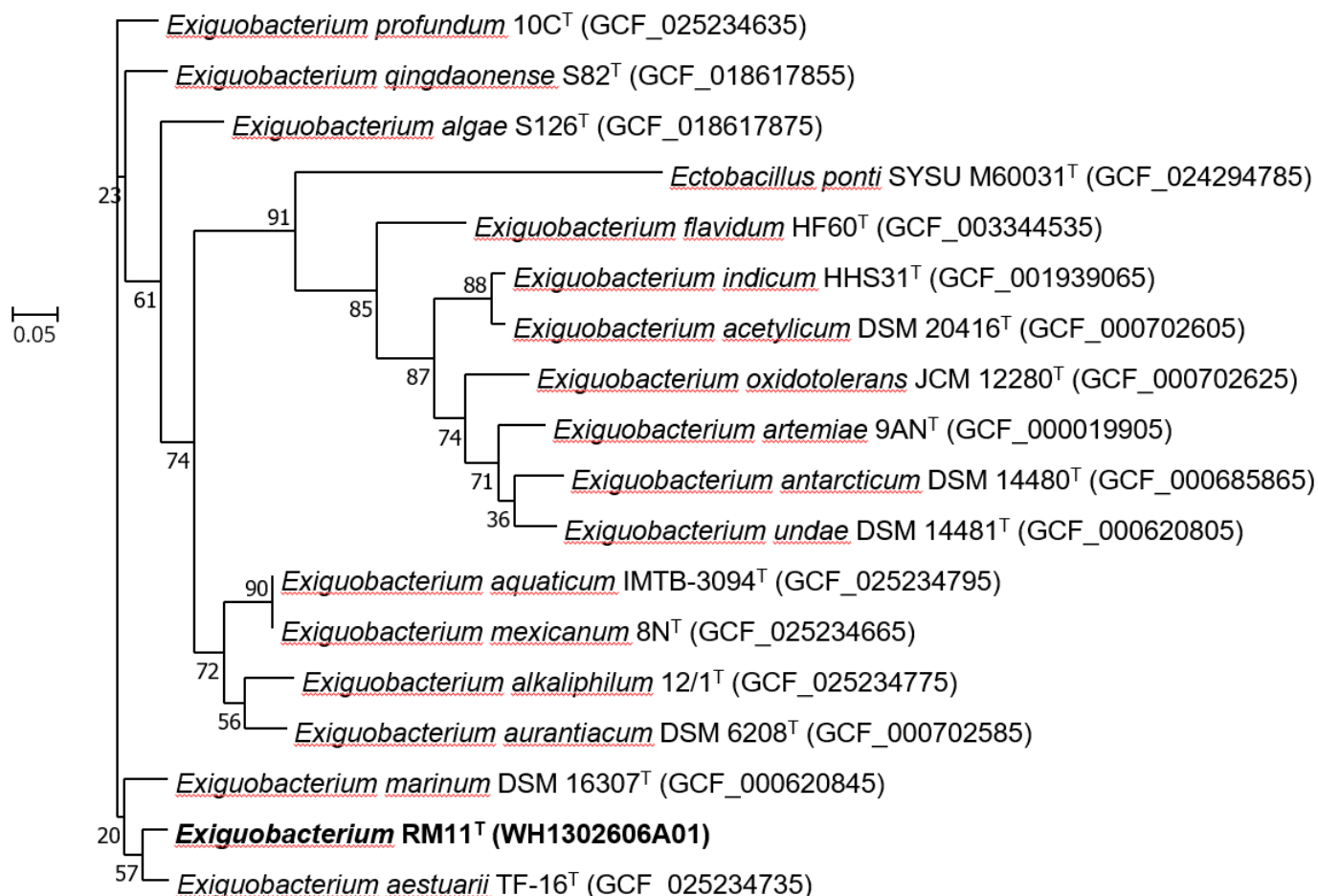


Figure 2

Genome-based phylogenetic tree of *Exiguobacterium* strain RM11^T and other related type strains using UBCGs (concatenated alignment of 92 core genes). Bootstrap values are indicated at nodes. Scale bar, 0.05 substitutions per position.

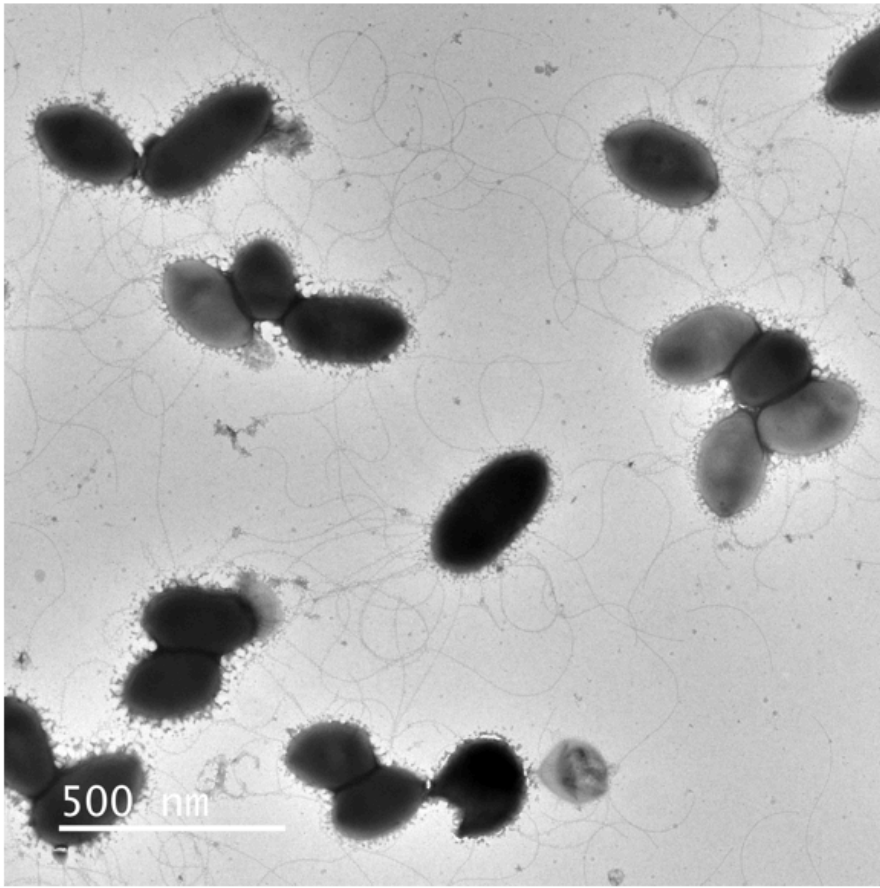
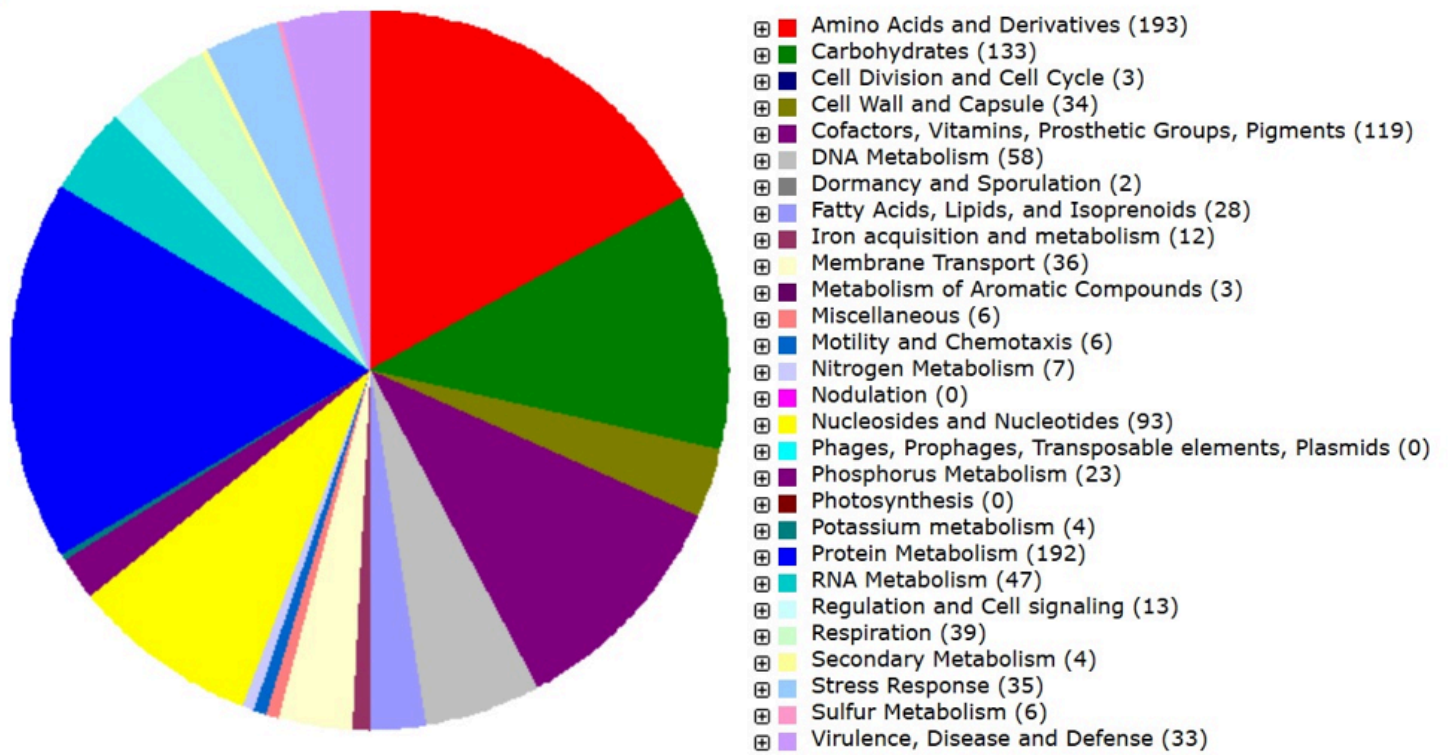


Figure 3

Transmission electron micrograph of RM11^T. Scale bar, 500 nm



The RAST annotation of RM11

Figure 4

RAST subsystem category distributions of novel *E. sargassi* RM11^T and the reference strains.

A, *Exiguobacterium sassgasi* RM11^T; B, *Exiguobacterium aestuarii* TF-16^T

C, *Exiguobacterium marinum* DSM 16307^T.

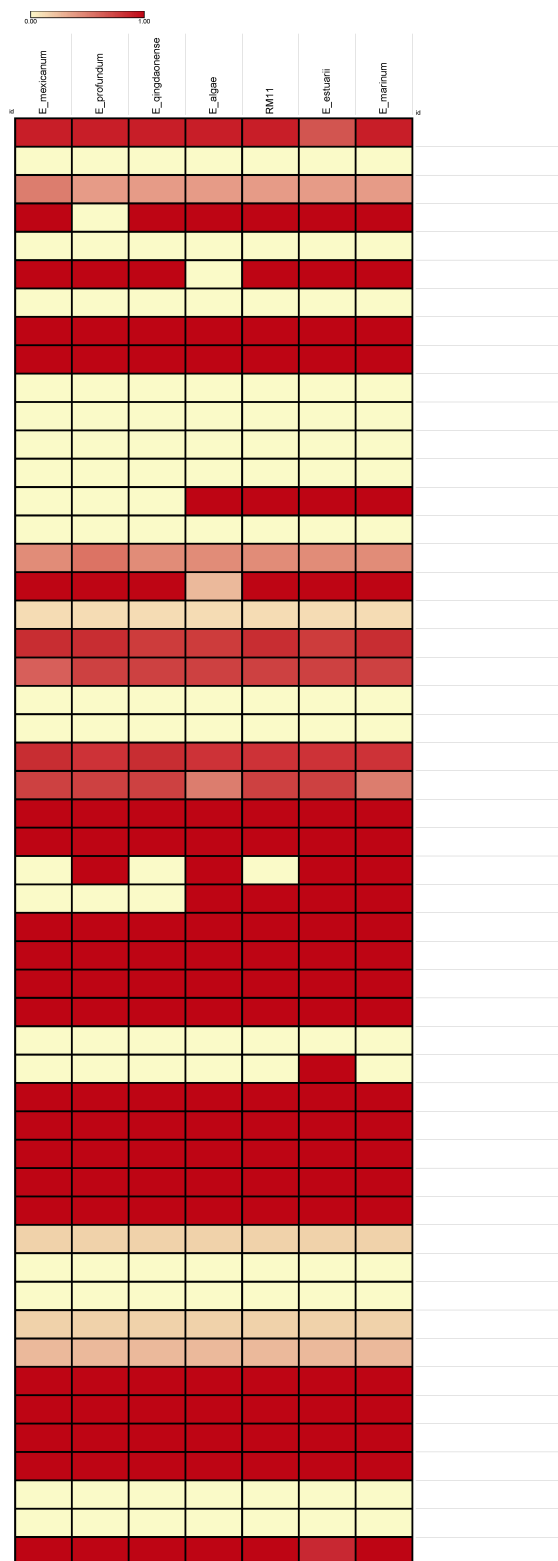


Figure 5

Heatmap showing the distribution of various metabolic pathways analyzed through KEGG analysis between the isolated strain RM11^T and related reference strains.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryDataS1RASTcomparison.xlsx](#)
- [supplementaryRM11.docx](#)