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Isolation and Complete Genome Sequence of *Algibacter alginolytica* sp. nov., a Novel Seaweed-Degrading *Bacteroidetes* Bacterium with Diverse Putative Polysaccharide Utilization Loci

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The members of the phylum *Bacteroidetes* are recognized as some of the most important specialists for the degradation of polysaccharides. However, in contrast to research on *Bacteroidetes* in the human gut, research on polysaccharide degradation by marine *Bacteroidetes* is still rare. The genus *Algibacter* belongs to the *Flavobacteriaceae* family of the *Bacteroidetes*, and most species in this genus are isolated from or near the habitat of algae, indicating a preference for the complex polysaccharides of algae. In this work, a novel brown-seaweed-degrading strain designated HZ22 was isolated from the surface of a brown seaweed (*Laminaria japonica*). On the basis of its physiological, chemotaxonomic, and genotypic characteristics, it is proposed that strain HZ22 represents a novel species in the genus *Algibacter* with the proposed name *Algibacter alginolytica* sp. nov. The genome of strain HZ22, the type strain of this species, harbors 3,371 coding sequences (CDSs) and 255 carbohydrate-active enzymes (CAZymes), including 104 glycoside hydrolases (GHs) and 18 polysaccharide lyases (PLs); this appears to be the highest proportion of CAZymes (~7.5%) among the reported strains in the class *Flavobacteria*. Seventeen polysaccharide utilization loci (PUL) are predicted to be specific for marine polysaccharides, especially algal polysaccharides from red, green, and brown seaweeds. In particular, PUL N is predicted to be specific for alginate. Taking these findings together with the results of assays of crude alginate lyases, we prove that strain HZ22^T can completely degrade alginate. This work reveals that strain HZ22^T has good potential for the degradation of algal polysaccharides and that the structure and related mechanism of PUL in strain HZ22^T are worth further research.

Members of the phylum *Bacteroidetes*, formerly also known as the *Cytophaga–Flavobacteria–Bacteroides* cluster, constitute one of the major groups of marine heterotrophic bacterioplankton (1, 2). They have been found in various marine habitats, including coastal sediments (3), coastal waters (4, 5), hydrothermal vents (6, 7), and open ocean waters (8–10). In previous studies, marine *Bacteroidetes* have been reported as important contributors to the utilization of biopolymers such as polysaccharides and proteins (2, 11–14). As a result, marine *Bacteroidetes* are assumed to play an important role in the degradation of algae. Marine phytoplankton have been estimated to be responsible for about 50% of global net primary production (15). Polysaccharides constitute a substantial fraction of the primary production from marine phytoplankton. Algae can be an important source of polysaccharides. Brown seaweeds, a traditional and plentiful mariculture product in East Asia, make up a large proportion of the total biomass of algae and synthesize a wide variety of compounds, such as alginate, fucoidan, laminarin, and mannitol (16). Among these compounds, alginate has been assumed to be a potential source for bioethanol production (17–19).

The genus *Algibacter* belongs to the family *Flavobacteriaceae* of the phylum *Bacteroidetes* and was first described by Nedashkovskaya et al. with the type species *Algibacter lectus* DSM 15365 (20). *Algibacter* species are Gram-negative, aerobic, nonmotile, pigmented bacteria that exhibit yellow to orange colonies. At the time of writing, according to the List of Prokaryotic Names with Standing in Nomenclature (21), there were 12 species in the genus *Algibacter*. *Algibacter* species have been isolated from seawater (22), green algae (20), intertidal sediment (23), marine sediment (24), sea urchins (25), and brown alga reservoirs (26–28). Most *Algibacter* species are isolated from or near the habitat of algae, indicating a preference for the complex polysaccharides of algae. To date, there has been only one genome announcement for *Algibacter*, about two draft genome sequences of *Algibacter lectus* strains SS8 and NR4 (29). Detailed analyses of the genome structures of *Algibacter* species and their relationships with algal polysaccharides are rare. Also, in marine *Bacteroidetes*, many strains have been sequenced with draft or complete genomes, but only for a few strains in the family *Flavobacteriaceae*, including *Zobellia galactanivorans* (30, 31), *Gramella forsetii* KT0803 (32, 33), *Formosa agariphila* KMM 3901^T (34), and *Polaribacter* sp. strains

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Hel1_33_49 and Hel1_85 (35), have the structure and function of polysaccharide utilization loci (PUL) been reported. The first report of PUL in marine *Bacteroidetes* was for *Zobellia galactanivorans*, which contains two PUL with alginate lyases that are induced by alginate (30). Later, PUL with similar structures were reported for *Gramella forsetii* KT0803, *Formosa agariphila* KMM 3901^T, and *Polaribacter* sp. strains Hel1_33_49 and Hel1_85. PUL are assumed to contain coregulated genes for the degradation of specified polysaccharides, with rapid and specific responses to polysaccharides; these operons can be significant for analysis of the spectrum of polysaccharides utilized by *Bacteroidetes* and for providing evidence of their ecological niche.

Previous genome analysis has revealed that in *Bacteroidetes*, the polysaccharide degradation genes, including those encoding carbohydrate-active enzymes (CAZymes) and transporters, are frequently carried in large operons or regulon structures called PUL; these structures have been reported in many *Bacteroidetes*, from commensal bacteria of the human intestine to marine organisms (32, 34, 36, 37). In short, PUL are clusters of coregulated genes or transcriptional units for polysaccharide degradation and related transportation. CAZymes are enzymes that degrade or modify carbohydrates or create glycosidic bonds with structurally related catalytic or carbohydrate-binding modules (CBMs); they include glycoside hydrolases (GHs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), glycosyltransferases (GTs), and CBMs (38). The CBMs are known as noncatalytic modules, adjacent to GHs or PLs, that bring the GHs or PLs into intimate association with their complex substrates (39). The transporters encoded in PUL are the outer membrane sugar-binding protein (SusD-like protein), a TonB-dependent receptor/transporter (TBDR; SusC-like protein) belonging to the bacteroidetal starch utilization system (Sus), and a related TonB-dependent transporter (TBDT) system, which can take up polysaccharide-degrading products into the cell (40, 41). In addition, most PUL contain genes encoding an inner membrane-associated sensor/regulator system, including the hybrid two-component systems (HTCSs) (36, 42). In *Bacteroidetes*, HTCSs are known as regulators, which mediate the rapid and specific responses of saccharides and control the expression of the PUL, and the depolymerized monosaccharide is the activating signal that binds directly to the periplasmic domain of the regulatory protein (36). An HTCS is composed of an N-terminal periplasmic sensor, which is interrupted by as many as 5 transmembrane segments, and 4 conserved cytoplasmic domains (histidine kinase [HK], phosphoacceptor domains [PD], a response regulator receiver [RR] domain, and a helix-turn-helix DNA-binding domain [HTH_AraC domain]) (42). The HTH_AraC domain in HTCSs is distinct from the HTH_8 domain, which is universal in classical two-component systems (43). Because of their conserved structure and coregulated genes, finding more PUL in marine *Bacteroidetes* will enhance our opportunities to analyze marine *Proteobacteria* and human gut *Bacteroides* that have the ability to degrade polysaccharides (44).

In our previous work (C. Sun, G. Fu, C. Zhang, and M. Wu, unpublished data), a novel *Algibacter* strain, HZ22, which could degrade brown seaweed, was isolated from the surface of a brown seaweed (*Laminaria japonica*). Based on the 16S rRNA gene sequence analysis, strain HZ22 was assumed to represent a novel species of the genus *Algibacter*. In order to analyze the phylogenetic affiliation of strain HZ22 and its mechanism of algal polysaccharide degradation, we identified strain HZ22 using polyphasic taxonomy methods, characterized its crude enzyme, sequenced its genome, and performed in-depth CAZyme and PUL analyses.

MATERIALS AND METHODS

Strain isolation and culture conditions. A brown seaweed sample was collected from the Zhoushan Islands (Zhejiang, China) in March 2014. The brown seaweed (*Laminaria japonica*) sample was first cut into pieces and then washed three times with autoclaved artificial seawater. The eluent was collected, diluted using a 10-fold series dilution method, and spread on seaweed artificial seawater medium. The seaweed artificial seawater medium contained the following (per liter of distilled water): brown seaweed powder, 10 g; NaCl, 23.4 g; $MgSO_4$, 12 g; $CaCl_2$, 2.9 g; KCl, 1.5 g (adjusted to pH 7.4 with NaOH). Twenty grams of agar per liter was added for solid medium. After 36 h of incubation at 28°C, one yellow colony was collected and was named HZ22. After repeated purification, the strain was routinely cultured on marine agar (MA; BD) at 28°C.

Phenotypic, chemotaxonomic, and genotypic characterization. The bacterium was tested for the Gram reaction by using the Gram-staining method (45). Cell morphology was examined by optical microscopy (BX40 microscope; Olympus) and transmission electron microscopy (JEM-1230 microscope; JEOL) using exponentially growing cells that were incubated on MA for 24 h. Growth at various NaCl concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, 10, and 12% [wt/vol]), a range of pHs (pH 5.0 to 10.5, with an interval of 0.5), and a range of temperatures (4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42, and 45°C) was carried out according to a protocol described previously (46). Catalase and oxidase were detected as described previously (45). Hydrolysis of hypoxanthine and xanthine was tested as described previously (47). Degradation of starch and L-tyrosine and hydrolysis of Tween 20, Tween 40, Tween 60, and Tween 80 were tested as described previously (45). Nitrate reduction, urease activity, and the ability to hydrolyze esculin, alginate, casein, chitin, carboxymethyl cellulose (CMC), filter paper, and gelatin were determined according to the method of Dong and Cai (48). The strain was tested for H_2S production and the methyl red and Voges-Proskauer reactions as described previously (49). Anaerobic growth was determined according to the method of Pan et al. (50). GN2 MicroPlates (Biolog, USA) were used to detect the utilization of organic substrates according to the manufacturer's instructions. Acid production was tested by using API 50CH (bioMérieux) strips. Leifson modified oxidative-fermentative (OF) medium (51) was used to suspend the cells for the inoculation of API 50CH strips. API 50CH strips were read after 24 h and 48 h. Additional physiological characteristics and enzyme activities were tested with API 20NE and API ZYM strips (bioMérieux), which were observed after 24 h and 4 h, respectively.

The cells for fatty acid methyl ester (FAME) analysis were incubated on MA at 28°C for 24 h and were analyzed according to the instructions of the Microbial Identification System (MIS; Microbial ID [MIDI], USA) with the standard MIS library generation software, version 4.5. The polar lipids were extracted, separated on silica gel 60 F_{254} plates (10 by 10 cm; Merck), and further analyzed as described previously (52, 53). Isoprenoid quinones were analyzed using reversed-phase high-performance liquid chromatography (HPLC) as described previously (54).

Genomic DNA was collected using the method of Marmur and Doty (55). The G+C content was determined by reversed-phase HPLC and was calculated from the ratio of deoxyguanosine (dG) to thymidine (dT) (56). The 16S rRNA gene was amplified and cloned into the pMD 19-T vector (TaKaRa) for sequencing (57). The complete 16S rRNA sequence of strain HZ22 was identified in the EzTaxon-e database (58) by using the EzTaxon-e tool. Phylogenetic trees were reconstructed by the neighbor-joining (NJ) (59) and maximum likelihood (ML) (60) methods with the MEGA 5 program package (61). According to the algorithm of the Kimura two-parameter model (62) for the neighbor-joining method, evolutionary distances were calculated with the MEGA 5 program package (61).

Enzyme assays. To collect the crude alginate lyases (cALY) of the isolate, experiments were carried out in a 250-ml flask (with 100 ml of seaweed artificial seawater medium) at 37°C and 140 rpm after 108 h. The

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fermentation products were centrifuged in triplicate at 12,000 rpm for 30 min to remove the cells and were then salted out with ammonium sulfate (20 to 80% saturation) and dialyzed with dialysis tubes (3.5 kDa; Spectrum) to remove useless compounds and ions. The alginate lyase (ALY) activity was determined using the dinitrosalicylic acid (DNS) method by quantitative estimation of the reducing sugars (63). Sodium alginate (Sigma) was used as the enzyme substrate, and D-glucuronic acid (Sigma) was used as the substrate for generation of the standard curve. Unless otherwise specified, the enzyme reaction was performed for 15 min at 40°C and pH 8.0, and the release of reducing sugars was measured at 540 nm using a UV-visible spectrophotometer (WFZ-UV2800H; Unico, China). All samples were measured in triplicate and were corrected for autohydrolysis of the substrate. One unit of enzyme activity was defined as the amount of enzyme that produced 1 μg of detectable reducing sugar at 40°C and pH 8.0 from the sodium alginate per min with glucuronic acid as the standard.

The effects of cations (Na^+, K^+, NH_4^+, Mg^{2+}, Cu^{2+}, Ca^{2+}, Fe^{2+}, Al^{3+}) and SDS on activity were examined at a final concentration of 1 mM. The effects of pH on activity and stability were measured at a pH range of 5.5 to 9.0, and stability was measured after 24 h. The effects of temperature on activity and stability were measured from 10 to 70°C, and stability was measured after 1 h. To detect substrate specificity, thin-layer chromatography (TLC) was carried out using the ascending method with a silica gel 60 F_{254} plate (20 by 20 cm; Merck). Sodium alginate (Sigma), polymannuronic acid, and polyguluronic acid were used as substrates. The mannuronic acid-rich fraction, as polymannuronic acid, and the guluronic acid-rich fraction, as polyguluronic acid, were prepared from sodium alginate after partial acid hydrolysis and were collected at different pHs (64). A solvent system of *n*-butanol–formic acid—water (4:6:1, by volume) was used to develop the decomposition products. Then the reaction products were visualized using the anisaldehyde reagent, followed by heating of the TLC plate at 120°C for 3 min (65, 66).

Genome sequencing, assembly, and annotation. DNA was extracted according to the method of Marmur and Doty (55). The genome was sequenced at the Beijing Genomics Institute (BGI) in Shenzhen, China, using Solexa paired-end sequencing technology (HiSeq 2000 system; Illumina, USA) (67). The shotgun library was constructed with a 500-bp paired-end library and a 6,000-bp mate-pair library. The resulting reads were filtered as follows: (i) removal of reads with a certain proportion of low-quality bases, (ii) removal of reads with a certain proportion of N′s, (iii) removal of adapter contamination, and (iv) removal of duplicate reads. A total of 2,610 Mbp of clean data, which provided ~140× coverage of the genome, was gathered. All clean reads were assembled using SOAPdenovo, version 2.04 (68). Thirty-three contigs (N_{50}, 679,161 bp; maximum length, 780,520 bp) were linked within 10 scaffolds (N_{50}, 2,960,049 bp; maximum length, 2,960,049 bp). Gaps were filled by multiplex PCR using high-fidelity PrimeSTAR DNA polymerase (TaKaRa), and the amplicons were sequenced by primer walking. After gap filling, a single complete circular chromosome (3,994,770 bp; G+C content, 31.8%) was obtained.

Coding sequences (CDSs) were predicted by using Glimmer, version 3.02 (69). tRNAs and rRNAs were identified using tRNAscan-SE (70), RNAmmer (71), and the Rfam database (72). The results from Glimmer were analyzed by the RAST (Rapid Annotations using Subsystems Technology) server (73). To verify the annotation, the predicted genes were searched against the NCBI NR protein database (74) and the Pfam (75), InterPro (76), COG (77), and KEGG (78) databases, and signal peptides and transmembrane regions were predicted by using SignalP, version 4.1 (79), and the TMHMM server, version 2.0 (80), respectively. All CAZymes were predicted by the CAZymes Analysis Toolkit (CAT) (81) and dbCAN HMMs, version 4.0 (82), using the CAZy database (38) and were then verified by reconstruction of phylogenetic trees based on the amino acid sequences of characterized CAZymes and manual annotation.

Nucleotide sequence accession number. The complete genome sequence of *Algibacter alginolytica* HZ22^T (JCM 18496; CGMCC 1.11025) has been deposited at the DDBJ/EMBL/GenBank database under accession number CP012898.

RESULTS AND DISCUSSION

Phenotypic, chemotaxonomic, and genotypic characterization. The physiological and biochemical characteristics of strain HZ22 are included in the species description below. A comparison of the physiological and biochemical characteristics of strain HZ22 and reference strains is shown in Table 1. For instance, strain HZ22 can grow at 4 to 55°C and 0 to 8% NaCl, higher temperature and NaCl concentration ranges than those of reference strains (35°C and 6% NaCl are the thresholds for most species of the genus *Algibacter*), indicating that strain HZ22 has an advantage in severe environments (20, 25, 26, 28). The hydrolysis of agar, esculin, starch, and L-tyrosine for strain HZ22^T contrasts with that of reference strains. The spectra of enzymes, such as esterase (C4), β-galactosidase, β-glucuronidase, and β-glucosidase, produced by strain HZ22 and reference strains also are not identical. In addition, the sole-carbon-source utilization results reveal that strain HZ22^T has different carbon source preferences (Table 1). With regard to the chemotaxonomic features that are important for distinguishing different genera, strain HZ22 shows no great differences from other *Algibacter* species. Detailed comparisons of fatty acid profiles and polar lipids for strain HZ22^T and reference strains are shown in Table S1 and Fig. S1 in the supplemental material, respectively. The similarities of 16S rRNA sequences in strain HZ22 with those of other strains in the genus *Algibacter* range from 94.8% to 97.2%, revealing that strain HZ22 may represent a novel species in this genus. The phylogenetic trees based on the 16S rRNA gene sequences, reconstructed with the NJ, ML, and maximum parsimony (MP) methods, show that strain HZ22 falls into the clade that comprises *Algibacter* species, forming a cluster with *A. lectus* KMM 3902^T, *Algibacter miyuki* WS-MY6^T, *Algibacter wandonensis* WS-MY22^T, and *Algibacter undariae* WS-MY9^T (Fig. 1). The genomic DNA G+C content of strain HZ22 is 31.8 mol%. This relatively low DNA G+C content is a feature of the genus *Algibacter* (<40%) (20, 26, 28). On the basis of its physiological, chemotaxonomic, and genotypic characteristics, it is proposed that strain HZ22 represents a novel species in the genus *Algibacter*, for which the name *Algibacter alginolytica* sp. nov. is proposed.

Enzyme assays of cALY. The optimum activity of cALY is measured over a pH range of 5.5 to 9.0 and a temperature range of 10 to 70°C with sodium alginate as the substrate. The optimum temperature for cALY activity is 40°C. But cALY are stable (>80%) at 20 to 30°C and relatively unstable at 40°C (~50%) or higher temperatures (50 to 70°C [<50%]) (Fig. 2a). cALY show the highest activity at pH 8.0 and retain a high level of activity (>80% of initial activity) at pH 6.5 to 9.0. However, measurement of the effect of pH on stability (24 h) shows that cALY are relatively stable (>50%) at pH 5.5 to 8.0, in contrast to pH effects on activity (Fig. 2b). The hydrolysis activity of cALY is increased in the presence of Na^+, reaching its maximum at 100 mM Na^+. cALY maintain their activity with most cations (1 mM) except for Ca^{2+}, and the addition of 0.1% SDS can inhibit cALY activity (Fig. 2c). The substrate specificity results indicate that cALY can hydrolyze sodium alginate, polymannuronic acid, and polyguluronic acid effectively (Fig. 3). With the increase of time, the polymeric levels of these three substrates are decreased rapidly, and polyguluronic acid is degraded more completely than the others (after 48 h).

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TABLE 1 Characteristics that differentiate strain HZ22^T from reference strains[a]

Characteristic	Strain HZ22^T	*A. lectus* KMM 3902^T	*A. miyuki* WS-MY6^T	*A. wandonensis* WS-MY22^T
Pigment	Yellow	Bright orange[b]	Yellow[c]	Vivid yellow[d]
NaCl concn (%) for growth (range [optimal])	0–8 (0.5–1.5)	1–6[b]	0–6 (2)[c]	0.5–6 (2)[d]
Temp (°C) for growth (range [optimal])	4–55 (28)	4–35 (21–23)[b]	4–35 (25)[c]	4–35 (25)[d]
Gliding motility	+	+	−	+
Voges-Proskauer test	+	−	−	−
H_2S production	−	+	+	+
Fermentation of glucose	−	+	−	−
Hydrolysis of:				
Agar	−	+	+	+
Esculin	−	+	+	+
Starch	+	−	−	−
L-Tyrosine	−	+	+	+
Production of:				
Esterase (C4)	w	w	w	+
β-Galactosidase	+	+	−	+
β-Glucuronidase	+	−	−	−
β-Glucosidase	−	+	+	+
Acid production from:				
Amygdalin	−	+	+	+
Gentiobiose	−	+	+	+
Inulin	−	+	+	+
D-Mannitol	−	w	−	+
D-Raffinose	−	+	+	+
D-Saccharose (sucrose)	−	+	+	+
Sole-carbon-source utilization				
Glycogen	−	+	+	+
D-Arabitol	+	−	−	−
Maltose	−	+	+	+
D-Psicose	+	−	−	−
D-Sorbitol	+	−	−	−
Sucrose	−	+	+	+
Succinic acid monomethyl ester	+	−	−	−
Itaconic acid	+	−	−	−
Succinic acid	+	−	−	−
D-Aspartic acid	−	+	+	−
D-Ornithine	−	+	+	+
DNA G+C content (mol%)	31.8	31.0–33.0[b]	35.3[c]	35.8[d]

[a] +, positive; −, negative; w, weakly positive. Unless stated otherwise, data were obtained from this study under identical growth conditions.
[b] Data from Nedashkovskaya et al. (20).
[c] Data from Park et al. (26).
[d] Data from Yoon and Park (28).

Genome properties. The complete genome sequence of strain HZ22^T reveals a genome of 3,994,770 bp with a G+C content of 31.8%, consisting of one single circular chromosome and no extra plasmid (Fig. 4). The genome contains 3,371 CDSs, to 2,327 of which predicted functions are assigned. The genome includes 40 tRNAs for all 20 standard amino acids and 2 copies of 16S–23S–5S rRNA gene operons located next to each other.

Energy metabolism. In the genome of strain HZ22^T, the Embden-Meyerhof-Parnas (EMP) pathway is intact. The genes encoding the pentose phosphate (PP) pathway, including the oxidative branch and the nonoxidative branch, are also intact. The Entner-Doudoroff (ED) pathway is incomplete, but the genes for 2-keto-3-deoxygluconokinase (KDGK; EC 2.7.1.45) and 2-keto-3-deoxyphosphogluconate aldolase (KDPGA; EC 4.1.2.14) are present. These two enzymes catalyze 2-keto-3-deoxygluconate (KDG) to 2-keto-3-deoxyphosphogluconate (KDPG) and KDPG to pyruvate, respectively. Strain HZ22^T may use the incomplete ED pathway to catalyze a monosaccharide of alginate to pyruvate and enter the Krebs cycle, as do other bacteria (83, 84). Moreover, the intact PP pathway reveals an important entrance for pentose-formed polysaccharides.

Strain HZ22^T has genes encoding both the pyruvate dehydrogenase complex (E1, E2, and E3) and pyruvate formate lyase for the conversion of pyruvate to acetyl coenzyme A. The Krebs cycle (also called the tricarboxylic acid [TCA] cycle) is intact but without the glyoxylate cycle. For electron transfer, genes encoding F_oF_1-type ATPase and the electron transfer chain (ETC), including complexes I, II, and IV but not complex III, are found. In

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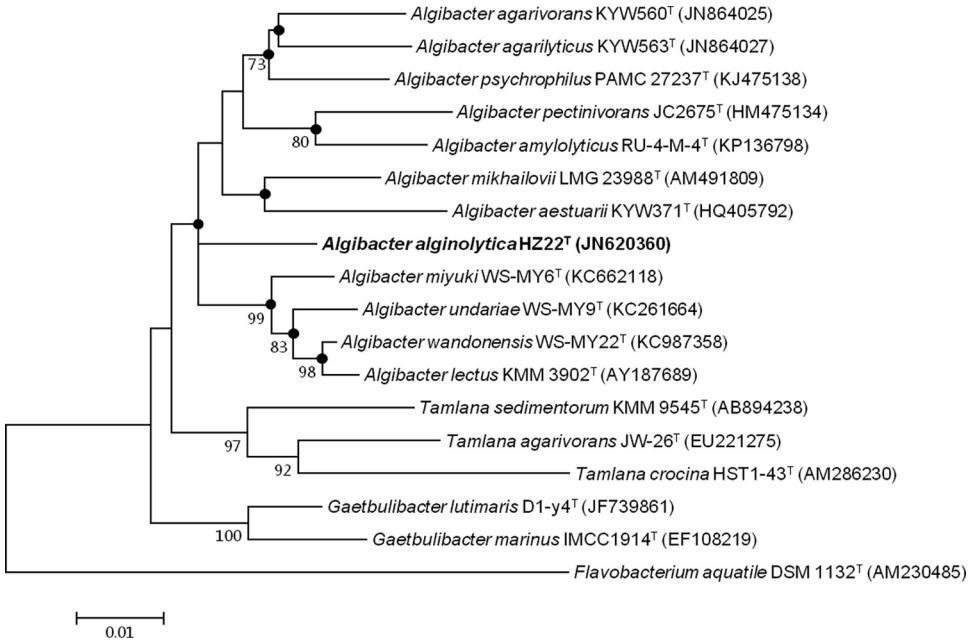

FIG 1 Neighbor-joining tree using the Kimura two-parameter model based on the 16S rRNA gene sequences of *Algibacter alginolytica* HZ22^T and related species. GenBank accession numbers are given in parentheses after strain names. Bootstrap values are based on 1,000 replicates; values higher than 70% are shown. Filled circles indicate nodes also obtained in both maximum likelihood and maximum parsimony trees. Bar, 0.01 substitution per nucleotide position. *Flavobacterium aquatile* DSM 1132^T (GenBank accession number AM230485) was used as the outgroup.

addition, according to the experimental results, the genome of strain HZ22^T shows no evidence of anaerobic growth. With regard to energy storage, the presence of genes encoding polyphosphate kinase (EC 2.7.4.1) and exopolyphosphatase (EC 3.6.1.11) indicates that strain HZ22^T may produce polyphosphate to store energy and phosphorus (34). For nitrogen utilization, a nitrate/nitrite transporter, assimilatory nitrate reductase, a two-component response regulator of nitrite reduction, and nitrate ABC transporter permease are annotated in strain HZ22^T.

Polysaccharide degradation. For polysaccharide degradation, CAZymes are the most important enzymes. On the basis of the CAZyme database, strain HZ22^T has 255 CAZymes, including 104 GHs, 18 PLs, 99 GTs, 29 CEs, and 13 CBMs (Fig. 4). The GHs and GTs belong to 29 and 13 families, respectively. The PLs are classified into seven families: families 1, 6, 7, 8, 10, 12, and 17. According to the statistics of Mann et al., the proportion of CAZymes in the genome is usually less than 5% for most bacteria that specialize in carbohydrate degradation (34). The proportion of CAZymes in strain HZ22^T is about 7.5%, which is quite high relative to those in most bacterial genomes (about 2%) and higher than those in most sequenced strains of the class *Flavobacteria*, e.g., *Formosa agariphila* KMM 3901^T (5.3%) and *Zobellia galactanivorans* Dsij^T (5.1%) (34, 85). The GHs and PLs are predicted for the degradation of the algal polysaccharides, including agarose, chitin, fucoidan (fucose-containing sulfated polysaccharides), fucoside (N-linked glycan), homogalacturonan, rhamnogalacturonan, starch (α-glucan), and xylan. Of these, agarose is a typical cell wall polysaccharide in red seaweed; the pectic compounds, such as homogalacturonan and rhamnogalacturonan, are found in the cell walls of several seaweeds; and xylan is found in the cell walls of plants, green algae, and red algae (86). The high proportion and wide substrate spectrum of CAZymes in the genome reveals that strain HZ22^T has good potential for degrading marine polysaccharides, especially algal polysaccharides in most seaweeds, including red, green, and brown seaweeds.

As indicated by the annotation, strain HZ22^T can degrade many polysaccharides with the predicted enzymes. The presence of alginate lyases (EC 4.2.2.3 and EC 4.2.2.11), including polymannuronic acid-specific, polyguluronic acid-specific, and polymannuronic acid- and polyguluronic acid-specific lyases and oligoalginate lyase in the PL6, -7, and -17 families, indicates that strain HZ22^T can degrade alginate into oligosaccharides or monosaccharides (87). The substrate specificity results reveal that cALY is capable of degrading polymannuronic acid, polyguluronic acid, and sodium alginate into small polymers (degree of polymerization [DP], about 2 to 5) (Fig. 3). With these DPs, oligoalginate can be transported though the outer membrane into the periplasmic space by TBDRs (32). According to all the results presented above, it is reasonable to posit that strain HZ22^T is capable of degrading alginate completely. The annotated β-agarase (GH16; EC 3.2.1.81) and β-galactosidase (GH2; EC 3.2.1.23) likely degrade agarose (88). β-Glucosidase (GH31; EC 3.2.1.21) and endo-1,4-β-glucanase (GH5; EC 3.2.1.4) are responsible for the degradation of cellulose (89). Chitin may be hydrolyzed by strain HZ22^T with chitinase (GH18; EC 3.2.1.14) and β-hexosaminidase (GH20; EC 3.2.1.52). The annotated α-L-fucosidases (GH29 and GH95; EC 3.2.1.51) can degrade fucosides and fucoidans (90). Two bifunctional enzymes (β-xylosidase/α-L-arabinofuranosidase [GH3]) are predicted to degrade arabinoxylan hemicelluloses (91). Homogalacturonan can be degraded via polygalacturonase (GH28; EC 3.2.1.15) and pectate lyases (PL1 and PL10; EC 4.2.2.2) (39). The predicted α-L-arabinofuranosidase (GH3; EC 3.2.1.55), unsaturated rhamnogalacturonyl hydrolase (GH105; EC 3.2.1.172), and α-L-rhamnosidase (GH78; EC

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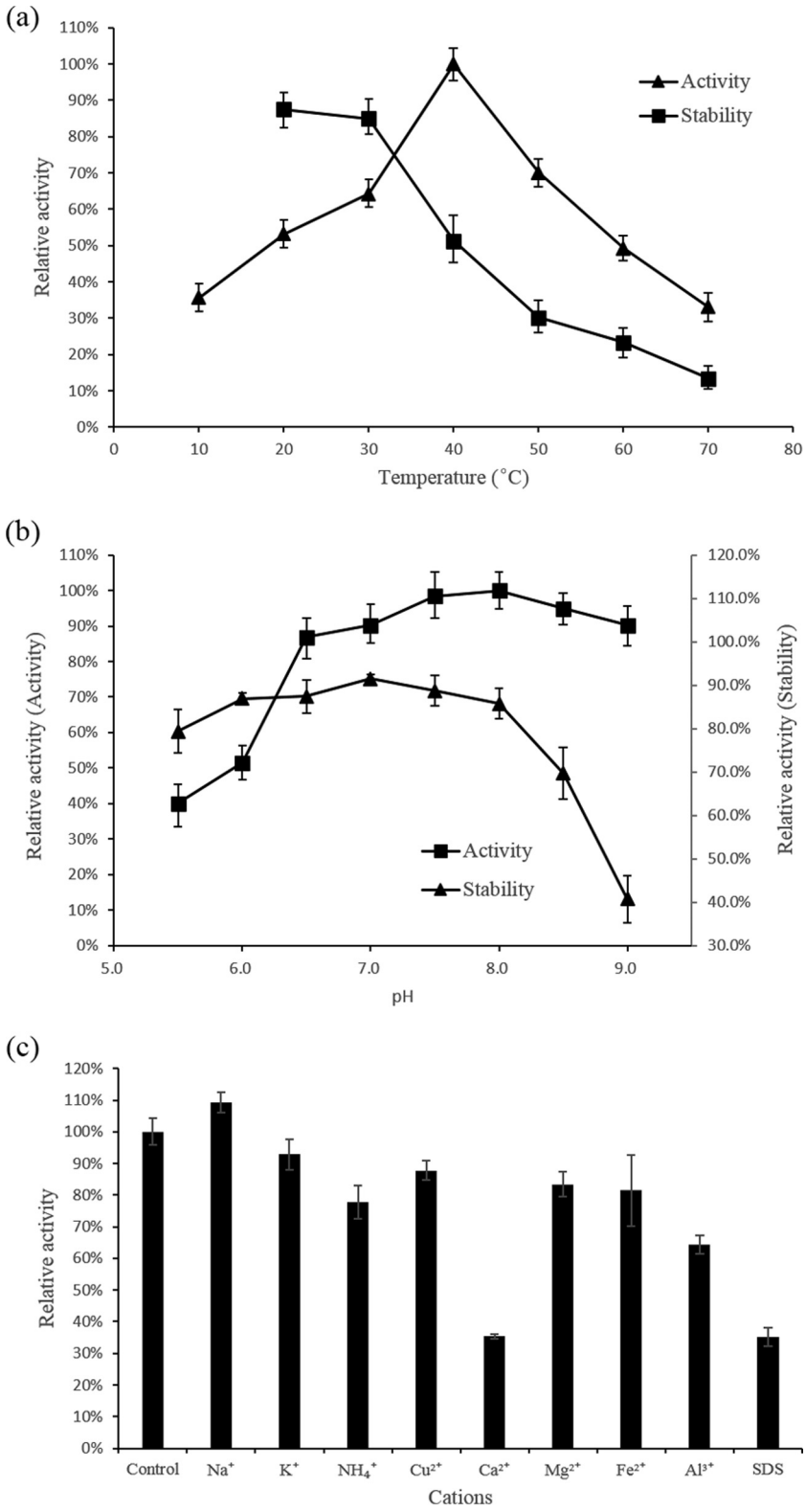

FIG 2 Biochemical properties of cALY. (a) Effects of temperature on the activity and stability of cALY. The activity value obtained at 40°C was taken as 100%. (b) Effects of pH on the activity and stability of cALY. The activity value obtained at pH 8.0 was taken as 100%. (c) Effects of cations and SDS on the activity of cALY. The value for the control with no additives in the reaction mixture was taken as 100%.

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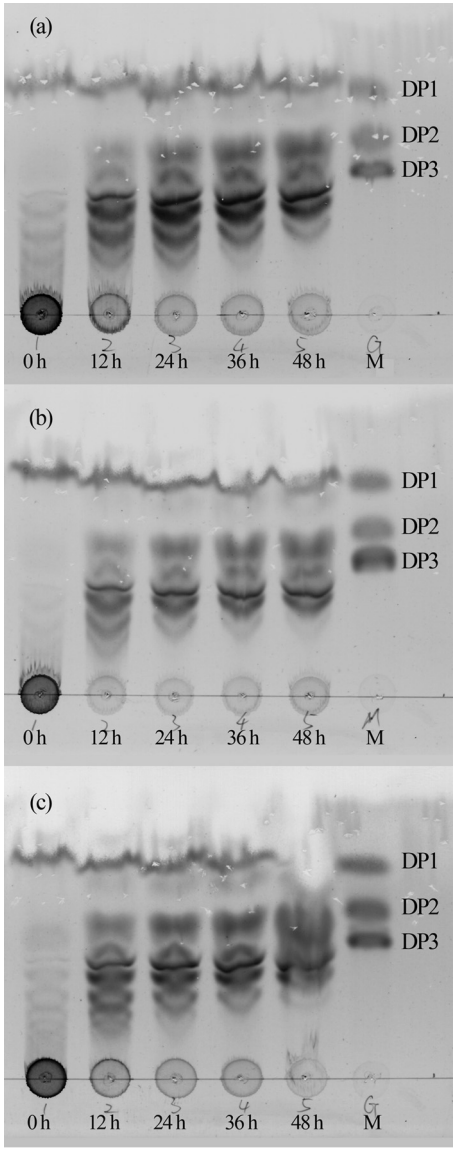

FIG 3 Thin-layer chromatograms of the products of sodium alginate, polymannuronic acid, and polyguluronic acid degradation by cALY, showing their substrate specificity. (a) Sodium alginate; (b) polymannuronic acid; (c) polyguluronic acid. DP, degree of polymerization.

3.2.1.40) may be used for the degradation of rhamnogalacturonan (39). Starch can be hydrolyzed with α-amylase (GH13; EC 3.2.1.1) and α-glucosidases (GH31; EC 3.2.1.20). The putative β-xylosidase (GH39 and GH43; EC 3.2.1.37), endo-1,4-β-xylanase (GH43; EC 3.2.1.8), and α-L-arabinofuranosidase (GH3; EC 3.2.1.55) are responsible for the degradation of xylan (37). In addition, strain HZ22^T has genes for the degradation of *N*-acetyl-β-D-hexosaminide residues, D-glucosyl-*N*-acylsphingosine residues, chondroitin sulfate-like mucopolysaccharides, and glycoproteins that are not polysaccharides but are also important in the degradation of algae (92).

Polysaccharide utilization loci. In this study, the defining criterion of a PUL is the presence of the predicted CAZymes for polysaccharide degradation and a gene pair encoding SusD-like protein and TBDR (36). According to this criterion, a total of 17 PUL are found in strain HZ22^T (Fig. 5). Moreover, 82 GHs (~80% of all GHs) and 18 PLs (100% of all PLs) are located in PUL. This high proportion of CAZymes in the PUL indicates that PUL are the key domains of most degradation reactions. The results of PUL function analysis show that most polysaccharides that are degradable by strain HZ22^T have corresponding PUL. In detail, PUL N is likely specific for alginate, PUL G for agarose, PUL P for cellulose, PUL L for chondroitin, PUL E for chitin, PUL A and PUL F for fucosides and fucoidans, PUL K for rhamnogalacturonan and homogalacturonan, PUL B for xanthan, and PUL C and PUL D for xylan. Among these PUL, the predicted structure of PUL N is quite different from that of PUL for alginate in other strains. PUL N encodes proteins that generally function in alginate degradation, such as alginate lyases (PL6, -7, and -17), KdgF-like protein, a GntR-like transcriptional regulator, a mannuronate transporter, and 4-deoxy-L-erythro-5-hexoseulose uronic acid (DEH) reductase. However, the enzymes that catalyze alginate monosaccharides into the ED pathway, i.e., KDPGA and KDGK, are absent in PUL N but are found in PUL Q, which contains only one GH but many enzymes for the utilization of monosaccharides. Hence, these predicted PUL suggest that the mechanism and regulation of alginate utilization in strain HZ22^T are different from those for other reported strains. Other predicted PUL are also important for the analysis of polysaccharide degradation and related transport and regulatory systems, and especially for metagenomic analysis in niche studies.

For sulfated polysaccharides, e.g., fucoidan, GHs are located with adjacent sulfatases in several PUL of strain HZ22^T (Fig. 5, PUL A, B, D, F, G, L, and O). A total of 51 sulfatases are found in strain HZ22^T, and 46 of these are located in PUL, indicating that sulfatases are important in polysaccharide degradation. The GHs colocated with sulfatases are α-L-fucosidase (PUL A, B, F, L, and O), β-glucosidase (PUL A), β-hexosaminidase (PUL A), α-1,2-mannosidase (PUL A), endo-1,4-β-xylanase (PUL G), and β-xylosidase (PUL F).

Transporters and regulators. The TBDT system, which comprises outer and cytoplasmic membrane components, is known for the transport of saccharides in PUL (40, 93). The components of the TBDT system function in the following steps: (i) SusD-like protein binds to oligosaccharides, which are degraded by secreted PLs or GHs; (ii) the TBDR transports oligosaccharides across the outer membrane via energy derived from the proton motive force and the TonB-ExbBD complex; (iii) the permeases/transporters of small saccharides on the cytoplasmic membrane transport the depolymerized saccharides into the cell (40, 94). In strain HZ22^T, 78 TBDRs are found, of which 28 are located in PUL and 50 are not, suggesting that the TBDT system in strain HZ22^T may have functions other than oligosaccharide transport. In addition, the finding of eight pairs of cytoplasmic membrane-localized anti-sigma factors and extracytoplasmic function (ECF) sigma factors in PUL of strain HZ22^T provide evidence of a regulatory function. These pairs, together with the TBDR and the TonB-ExbBD complex, are also known as TonB-dependent regulatory systems. Such systems accept signals from outside the bacterial cell and transmit them into the cytoplasm, leading to the transcriptional activation of target genes (95). Therefore, the TBDT systems in strain HZ22^T PUL may function as regulators as well as transporters.

With regard to the regulators, strain HZ22^T encodes 9 HTCSs, 7 of which are located in PUL (Fig. 5, PUL C, F, I, J, K, M, and P). This result reveals that the HTCS, as in human gut *Bacteroidetes*, is

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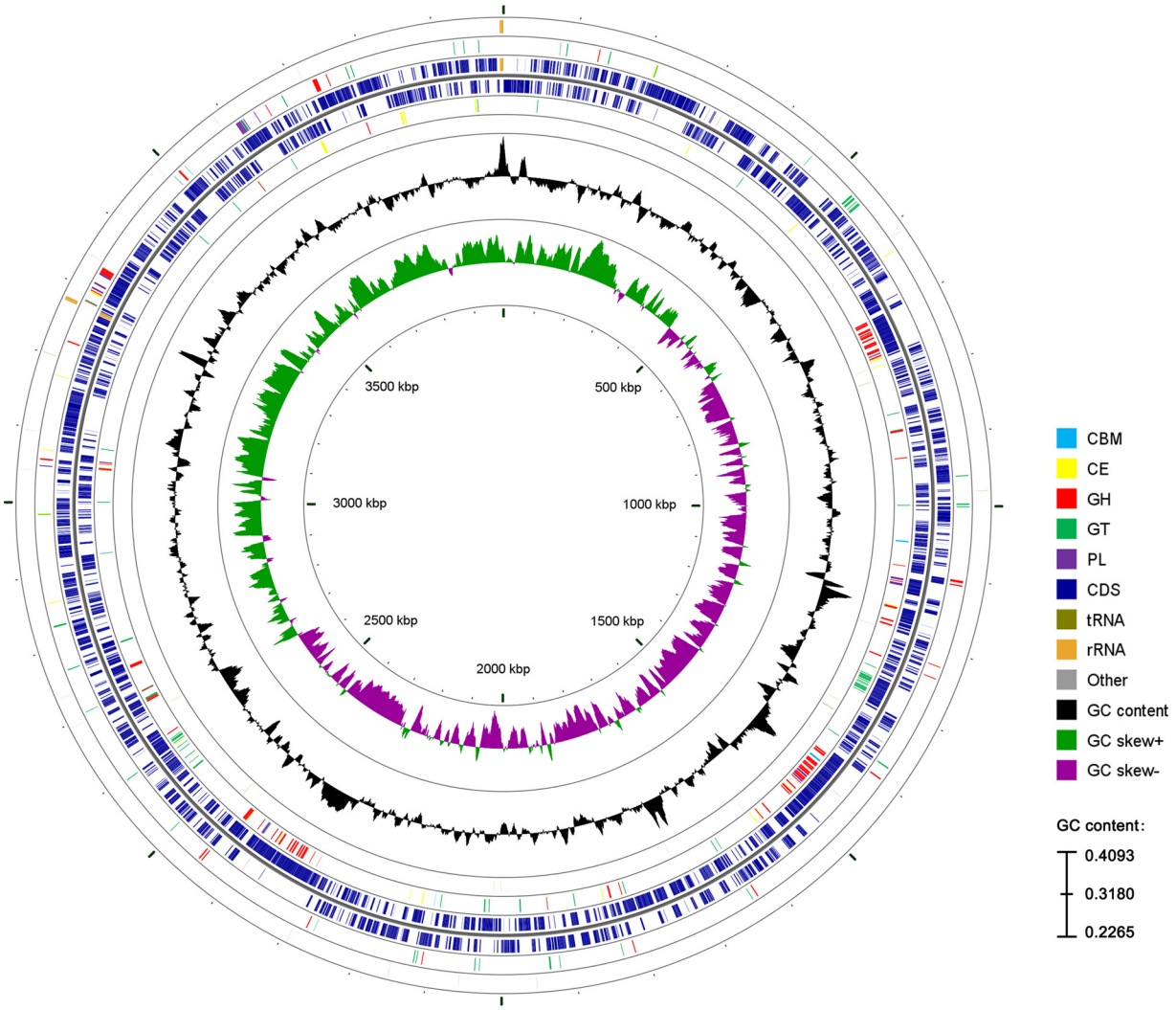

FIG 4 Schematic circular representation of the complete genome of *Algibacter alginolytica* HZ22^T. Note the following features (with circles numbered from the innermost [first circle] to the outermost [ninth circle]): RNAs on forward and reverse chains (ninth and fourth circles), CAZymes on forward and reverse chains (eighth and fifth circles), CDSs on forward and reverse chains (seventh and sixth circles), GC content (third circle), GC skew (second circle), sequence address in nucleotides (first circle). The circular representation was computed and drawn by the CGView program with an application programming interface (API) (96) and self-written PERL scripts.

the regulator for polysaccharide degradation in marine *Bacteroidetes*. In addition, 11 single DNA-binding response regulators (adjacent to the HK, PD, and RR, but not in one polypeptide) in AraC or LuxR families and 16 other classical two-component response regulators are annotated. Thus, the classical two-component systems may also function as regulators in PUL of strain HZ22^T. The relationships between these three regulatory systems for polysaccharide degradation, and their coregulatory mechanisms, need further study.

Besides the transporters and regulators for polysaccharide degradation, a total of 95 transporters and 74 predicted transcriptional regulators with different substrates are annotated. In detail, the following ion transporters are predicted: ammonium, chromate, cobalt, and ferrous iron transporters, a lead, cadmium, zinc, and mercury transporter, a magnesium and cobalt transporter, a magnesium, cobalt, and nickel transporter, manganese and molybdenum transporters, a nitrate and nitrite transporter, and phosphate, potassium, sulfate, and zinc transporters. For organic compounds, *N*-acetylglucosamine, di-/tripeptide, drug/metabolite, formate, mannuronate, preQ0, RND (resistance-nodulation-division) multidrug efflux, and thiamine transporters are predicted. Thirty ATP-binding cassette (ABC) transporters for copper, dipeptide, iron, lipopolysaccharide, methionine, manganese, nitrate, phosphonate, and vitamin B_{12} are annotated. Twelve sodium-coupled symporters/exchangers, including a bile acid symporter, calcium exchanger, mannose transporter, galactose transporter, hydrogen exchanger, iodide cotransporter, and solute symporter, are annotated. Seventy-four transcriptional regulators are predicted within the AsnC, AraC, ArsR, Cro/Cl, Crp/Fnr, CytR, GntR, HxlR, LacI, LuxR, MarR, MecI, MerR, MntR, PadR, Rrf2, TetR, XRE, and ZraR families.

The isolation and genomic analysis of strain HZ22^T represent initial work on polysaccharide degradation in marine *Bacte-*

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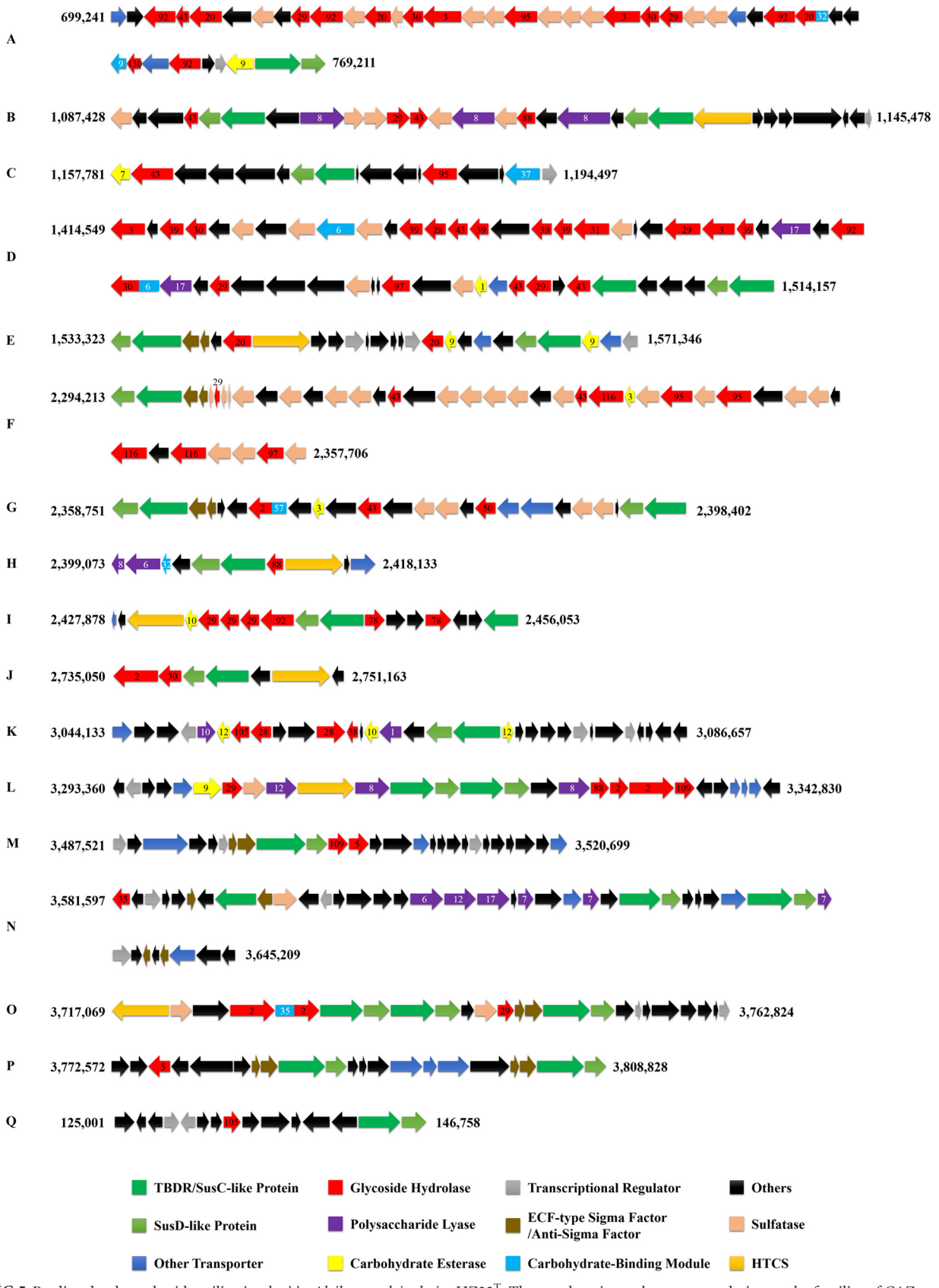

FIG 5 Predicted polysaccharide utilization loci in *Algibacter alginolytica* HZ22^T. The numbers in or above arrows designate the families of CAZymes.

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roidetes, about which we want to know more. On the basis of this work, further studies of the mechanism of polysaccharide degradation and its related transport and regulation systems in PUL will be able to proceed.

Description of *Algibacter alginolytica* sp. nov. *Algibacter alginolytica* (al.gi.no.ly′.ti.ca. N.L. n. *acidum alginicum*, alginic acid; N.L. fem. adj. *lytica* [from Gr. fem. adj. *lutikê*], able to dissolve; N.L. fem. adj. *alginolytica*, alginic acid dissolving).

Cells of strain HZ22^T are aerobic Gram-negative short rods (0.1 to 0.3 by 1.0 to 1.4 μm) with gliding motility. Colonies on Marine Agar 2216 plates are yellow, circular, convex, and smooth, with a diameter of 1.0 to 2.0 mm, after incubation at 28°C for 5 days. Growth is observed at 4 to 55°C, pH 5.5 to 8.5, and 0 to 8% (wt/vol) NaCl. Optimal growth is observed at 28°C, pH 7.0 to 7.5, and 0.5 to 1.5% (wt/vol) NaCl. No anaerobic growth is observed. Carotenoid pigment is produced. Flexirubin pigment is absent. Catalase and oxidase tests are positive. The strain is positive by the Voges-Proskauer test and negative for H_2S production. Tween 20, Tween 40, Tween 60, Tween 80, starch, gelatin, and alginate are hydrolyzed, but not agar, CMC, filter paper, casein, tyrosine, xanthine, or hypoxanthine. The predominant respiratory quinone is menaquinone-6. The major fatty acids are iso-$C_{15:0}$ (34.4%), iso-$C_{15:1}$ G (15.6%), anteiso-$C_{15:0}$ (8.3%), $C_{15:0}$ (7.2%), and ECL 13.565 (9.3%). The main polar lipids are phosphatidylethanolamine (PE), an unknown aminolipid (AL), and three unknown lipids (L). Strain HZ22^T is susceptible to lincomycin, tetracycline, carbenicillin, chloramphenicol, cephalothin, penicillin G, and ampicillin but not to polymyxin B, streptomycin, kanamycin, neomycin, gentamicin, or novobiocin. In the API 20NE test, the strain is positive for β-galactosidase activity and hydrolysis of gelatin and negative for glucose fermentation, nitrate reduction, indole production, arginine dihydrolase and urease activities, and hydrolysis of esculin. In the API ZYM test, strain HZ22^T is positive for alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-glucuronidase, esterase lipase (C_8), β-galactosidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, and β-fucosidase activities, has weak esterase (C_4) activity, and is negative for lipase (C_{14}), cystine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucosidase, and α-mannosidase activities. In API 50CH tests, acid is produced from D-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, *N*-acetylglucosamine, D-cellobiose, D-lactose (of bovine origin), and L-fucose but not from other substrates in the strip. In the Biolog GN2 test, growth using the following substrates (but not other substrates in the strip) as the sole carbon source is observed: *N*-acetyl-D-glucosamine, adonitol, D-arabitol, D-cellobiose, D-fructose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, D-psicose, D-raffinose, D-sorbitol, sucrose, turanose, succinic acid monomethyl ester, D-glucuronic acid, β-hydroxybutyric acid, itaconic acid, α-ketovaleric acid, D-saccharic acid, succinic acid, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-ornithine, L-pyroglutamic acid, uridine, α-D-glucose-1-phosphate, and D-glucose-6-phosphate. The G+C content of genomic DNA is 31.8 mol%.

The type strain is HZ22^T (CGMCC 1.11025^T; JCM 18496^T), isolated from the surface of a brown seaweed (*Laminaria japonica*) in Zhejiang, China.

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