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Characterization of *Kordiimonas marina* sp. nov. and *Kordiimonas laminariae* sp. nov. and Comparative Genomic Analysis of the Genus *Kordiimonas*, A Marine-Adapted Taxon

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Two novel rod-shaped and Gram-negative bacterial strains, designated A6E486^T and 5E331^T, were isolated from a coastal sediment sample taken from Xiaoshi Island, Weihai, China, and a fresh kelp sample collected from a kelp culture area, Rongcheng, China, respectively. Growth of strain A6E486^T occurred at 20°C–43°C (optimum, 33°C–35°C) at pH 5.5–7.5 (optimum, 6.5) and in the presence of 1.0%–5.5% (w/v) NaCl (optimum, 2.5%–3.0%). Strain 5E331^T grew with 1.5%–5.0% (w/v) NaCl (optimum, 3.0%) at 15°C–40°C (optimum, 33°) and pH 6.0–8.5 (optimum, 7.0). The similarity of 16S rRNA gene sequence between the two strains was 95.2%. The phylogenetic analysis based on 16S rRNA gene sequence showed that strains A6E486^T and 5E331^T belong to the genus *Kordiimonas*, sharing the highest similarity to the genus *Kordiimonas* (94.6%–96.8%, 94.9%–96.1%, respectively). Strains A6E486^T and 5E331^T had percentage of conserved protein (POCP) values of 56.0%–67.3% and average nucleotide identity (ANI) values of 68.8%–73.1% to members of the genus *Kordiimonas*. The major polar lipids detected in the two strains were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and unidentified glycolipids, aminolipids, and lipids. The predominant respiratory quinone of the two strains was Q-10. Based upon the results presented in this study, strains A6E486^T and 5E331^T represent two novel species of the genus *Kordiimonas*, for which the names *Kordiimonas marina* and *Kordiimonas laminariae* are proposed with the type strains A6E486^T (= KCTC 82758^T = MCCC 1H00470^T) and 5E331^T (= KCTC 92199^T = MCCC 1H00515^T), respectively. Comparative genomic analysis showed that seven species of the genus *Kordiimonas* shared 1,258 core genes and had differences in carbohydrate metabolism, energy metabolism, and cofactor and vitamin metabolism. The pan-genome of the genus *Kordiimonas* was open. The prediction of secondary metabolites showed that most strains of the genus *Kordiimonas* had the ability to produce homoserine lactones, one of the most important

signal molecules in the quorum-sensing system of Gram-negative bacteria. Additionally, numerous genes involved in bacterial defense, motility and chemotaxis, cold adaptation, and environment stress response were found in the genus *Kordiimonas*, indicating the marine-adapted lifestyle of members of the genus *Kordiimonas*.

Keywords: *Kordiimonas*, polyphasic taxonomy, comparative genomic analysis, metabolic pathways, homoserine lactone, marine-adapted lifestyle

1 INTRODUCTION

The genus *Kordiimonas* was firstly proposed as a member of the order *Kordiimonadales* in the class *Alphaproteobacteria* by Kwon et al. (2005), with *Kordiimonas gwangyangensis* being the type species. At the time of writing, the genus *Kordiimonas* consists of seven validly published species according to the List of Prokaryotic names with Standing in Nomenclature[1], including *K. gwangyangensis*, *Kordiimonas lacus*, *Kordiimonas aestuarii*, *Kordiimonas aquimaris*, *Kordiimonas lipolytica*, *Kordiimonas sediminis*, and *Kordiimonas pumila*. All members of the genus *Kordiimonas* are aerobic, oxidase- and catalase-positive, except *K. sediminis* (facultatively anaerobic, oxidase- and catalase-negative) (Zhang et al., 2016). All of the type strains of the genus *Kordiimonas* are isolated from marine environments and habitats, such as marine sediment (Kwon et al., 2005; Xu X. et al., 2011; Math et al., 2012; Zhao et al., 2018), seawater (Yang et al., 2013; Wu et al., 2016), and sea cucumber culture pond sediments (Zhang et al., 2016), and have Q-10 as the predominant respiratory quinone. In this study, we describe two bacterial strains, A6E486^T isolated from a coastal sediment sample and 5E331^T isolated from a fresh kelp sample. Polyphasic investigations showed that the two strains represent two novel species affiliated with the genus *Kordiimonas*. Comparative genomic analyses of these two strains and the related species are contributed to understand the marine-adapted lifestyle of the genus *Kordiimonas*.[1]

2 MATERIALS AND METHODS

2.1 Bacterial Isolation and Cultivation

Strain A6E486^T was isolated from an intertidal sediment sample collected from Xiaoshi Island (37°32'30"N, 122°6'20"E), one of the largest continuous ecosystems in Weihai, China. The sediment sample was treated with an enrichment culture technique at 33°C for 30 days (Mu et al., 2018), and 100-μl enrichment cultures were spread on marine agar 2216 (MA; BD) by using the standard 10-fold dilution plating technique. After incubation at 33°C for 7 days, a beige colony designated A6E486^T was picked and pure cultures were obtained using purification procedures. Strain 5E331^T was obtained from fresh kelp samples, which were taken from a kelp culture area (37°15'96"N, 122° 36'49"E) in Rongcheng, China. Here, 10 g of fresh kelps were cut into small squares with sides of 1.5 cm under sterile conditions. Then, treated samples were placed in a conical flask containing glass beads and 100 ml seawater and shaken at 300 rpm for 1.5 h. Subsequently, the kelp treatment fluid was serially diluted to 10^{-3} with sterile seawater, and 100 μl aliquot was spread on MA. The incubation was performed at 28°C for 2 weeks. Strains A6E486^T and 5E331^T were stored at −80°C in sterile 1% (w/v) saline supplemented with 15% (v/v) glycerol. The type strains *K. gwangyangensis* JCM 12864^T and *K. lipolytica* JCM 30877^T obtained from the Japan Collection of Microorganisms were used as experiment control strains and cultured under optimum conditions.

2.2 Morphological, Physiological, and Biochemical Characteristics

The morphological and physiological features of strains A6E486^T and 5E331^T were examined after incubation at 33°C for 3 days on MA medium. The Gram staining reaction was determined according to the method described previously (Dong and Cai, 2001). Cell morphology and size were examined employing optical microscopy (E600, Nikon), transmission electron microscopy (JEM-1200, Jeol), and scanning electron microscopy (model Nova NanoSEM450, FEI). Growth ranges and optima of temperature were determined on MA at 0°C, 4°C, 15°C, 20°C, 25°C, 28°C, 30°C, 33°C, 35°C, 37°C, 40°C, 43°C, 45°C. The pH range for growth was tested in marine broth 2216 (MB; BD) by adding the appropriate buffers (20 mM), including 2-Morpholinoethanesulfonic acid (MES) (pH 5.5–6.0), 1,4-Piperazinediethanesulfonic acid (PIPES) (pH 6.5–7.0), N-(2-Hydroxyethyl) piperazine-N'-2-ethanesulfonic acid (HEPES) (pH 7.5–8.0), Tricine (pH 8.5), and 3-(Cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO) (pH 9.0–9.5). The optimal condition with NaCl for growth was tested in the following medium (0.1% yeast extract, 0.5% peptone), prepared with artificial seawater (per liter: 3.2 g $MgSO_4$, 2.2 g $MgCl_2$, 1.2 g $CaCl_2$, 0.7 g KCl, 0.2 g $NaHCO_3$) containing NaCl at concentrations from 0% to 10% (w/v, in 0.5% intervals). The strains were incubated at optimum temperature for 7 days. Growth experiments with leucine as sole carbon source were tested in the following liquid media, artificial seawater with inorganic carbon ($NaHCO_3$) removed was supplemented with

Abbreviations: ANI, average nucleotide identity; POCP, percentage of conserved proteins; KEGG, Kyoto Encyclopedia of Genes and Genomes; MCCC, Marine Culture Collection of China; KCTC, Korean Collection for Type Cultures; JCM, Japan Collection of Microorganisms; NCBI, National Center for Biotechnology Information; PGAP, Prokaryotic Genome Annotation Pipeline; HPLC, high-performance liquid chromatography; NJ, neighbor-joining; ML, maximum-likelihood; MP, maximum-parsimony; Pfam, Protein Families Database; GO, Gene Ontology; RAST, Rapid Annotation using Subsystem Technology; BPGA, Bacterial Pan Genome Analysis.

[1] https://www.bacterio.net/species

0.1% NH_4Cl as a nitrogen source with or without leucine. Leucine solution was sterilized with 0.22-μm filters. The final concentration of leucine in the medium was 1 g/L. The strains were incubated at optimum temperature for 11 days. Bacterial growth was monitored using a spectrophotometer at 600 nm.

Catalase activity was tested by adding 3% (v/v) H_2O_2 solution to the plate with fresh colonies. Oxidase activity was determined using an oxidase test reagent (bioMérieux). Anaerobic growth was determined by incubating strains on MA with or without 0.1% (w/v) KNO_3 in an AnaeroPack for 14 days. Hydrolysis tests of Tweens (20, 40, 60, and 80), alginate, casein, CM-cellulose, DNA, and starch were carried out according to methods described previously (Dong and Cai, 2001). The API 50CH and API 20E kits (bioMérieux) were employed to investigate acid production and additional biochemical characteristics, respectively. Additional enzyme activities were checked using API ZYM kit (bioMérieux), and carbon source oxidation was tested employing BIOLOG GEN III MicroPlates. All of the API and BIOLOG tests were carried out according to the manufacturer's instructions, except for the modification of adjusting salinity to 3% (w/v). Antibiotic susceptibility was determined on MA at 33°C for 7 days using the disc diffusion method (Jorgensen and Turnidge, 2015).

2.3 Chemotaxonomic Properties

Strains A6E486^T and 5E331^T and experiment control strains were cultured in MB under optimum conditions, and the cells at the late stage of exponential growth phase were harvested and freeze-dried in order to investigate chemotaxonomic characterizations. Fatty acids were extracted from freeze-dried cell biomasses (10 mg) and then analyzed using a gas chromatograph (model 6890N, Agilent) and the Microbial Identification System (MIDI database: TSBA40) (Sasser, 1990). Isoprenoid quinones were obtained from freeze-dried cell biomasses (300 mg) and identified by high-performance liquid chromatography (HPLC), as described previously (Minnikin et al., 1984). Total lipids were extracted with chloroform/methanol/water system (2.5:5:2, v/v/v) and analyzed by two-dimensional TLC according to the modified method described previously (Komagata and Suzuki, 1987).

2.4 16S rRNA Gene Sequence and Phylogenetic Analysis

The 16S rRNA gene was amplified employing PCR technology with the primer pair 27F and 1492R (Lane, 1991) and was sequenced by RuiBiotech (Qingdao, China). To obtain nearly complete 16S rRNA genes, purified PCR products were ligated into the pMD18-T vector (Takara), and ligation products were transferred into *Escherichia coli* DH5a receptor cells according to the manufacturer's instructions. For comparative analysis, the 16S rRNA gene sequences of strains A6E486^T and 5E331^T were submitted to the National Center for Biotechnology Information (NCBI) database[2] and the EzBioCloud server.[3]

The 16S rRNA gene sequences of strains A6E486^T, 5E331^T, and relatives were aligned using MUSCLE (Edgar, 2004), and then the trimmed sequences were used to reconstruct phylogenetic trees employing the neighbor-joining (NJ) and maximum-parsimony (MP) algorithm implemented in the software MEGA X (Felsenstein, 1981; Saitou and Nei, 1987; Kumar et al., 2018). The maximum-likelihood (ML) tree was reconstructed using the best-fit substitution model GTR+G+I. Bootstrap analysis was performed with 1,000 replications to provide confidence estimates for tree topologies.

2.5 Genomic Analysis

Genomic DNA of strains A6E486^T and 5E331^T was extracted using a bacteria genomic DNA kit (Takara), and the draft genomes were determined by Beijing Novogene Bioinformatics Technology Co. Ltd. (Beijing, China). Sequencing was performed on the Illumina NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA) using the paired-end 150-bp sequencing protocol. Raw sequencing data were generated by Illumina base-calling software CASAVA v1.8.2[4], and the sequenced reads were assembled using SOAPdenovo software (Li et al., 2009). Other relevant genomes in this study were obtained from the NCBI Prokaryotic reference genome database.

The completeness values of the used genomes were estimated based on the method of lineage-specific CheckM v1.1.3 (Parks et al., 2015). Gene content was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The average nucleotide identity (ANI) was calculated using the online ANI calculator[5]. The percentage of conserved protein (POCP) value was calculated as described by Qin et al. (2014).[6] The ribosomal protein phylogenetic tree was reconstructed by FastTree (Price et al., 2009) using JTT+ CAT parameters and IQ-TREE (Nguyen et al., 2015; Chernomor et al., 2016) using the LG +F+I+G4 model. The 15 ribosomal proteins (L2, L3, L4, L5, L6, L14, L15, L16, L22, L24, S3, S8, S10, S17, and S19) were obtained and aligned using Python script[6] with the methods described by Wiegand et al. (2020). All genomes were predicted using Prodigal Server for uniformity (Hyatt et al., 2010). The analysis of core and unique genes was performed using the Bacterial Pan Genome Analysis (BPGA) tool, an ultrafast pan-genome analysis pipeline, with a threshold of 50% amino acid sequence identity (Chaudhari et al., 2016). Metabolic pathways were analyzed in detail by using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Kanehisa et al., 2016), and the presence of gene clusters encoding secondary metabolites was predicted by antiSMASH server[7]. Carbohydrate-active enzymes of the genus *Kordiimonas* were annotated employing the online dbCAN2[8], a meta server for automated carbohydrate-active enzyme annotation (Zhang et al., 2018). The analysis of marine adaptation metabolic features was performed by Rapid Annotation using Subsystem Technology (RAST) version 2.0 (Aziz et al., 2008) and the KEGG database (Kanehisa et al., 2016).

[2] https://blast.ncbi.nlm.nih.gov/Blast.cgi
[3] http://www.ezbiocloud.net/
[4] http://support.illumina.com/
[6] https://github.com/2015qyliang/BGAStudio
[7] https://antismash.secondarymetabolites.org/#!/start

3 RESULTS AND DISCUSSION

3.1 Morphological, Physiological, and Biochemical Characteristics

Morphological observations by transmission electron microscopy and scanning electron microscopy showed that cells of strains A6E486^T (with widths of 0.5–0.7 μm and lengths of 1.0–2.5 μm) and 5E331^T (with widths of 0.2–0.5 μm and lengths of 0.8–2.7 μm) were both short rod-shaped (**Supplementary Figure S1**). For strain A6E486^T, the oxidations of the sole carbon source were positive for D-maltose, D-trehalose, D-fructose, glycyl-L-proline, L-glutamic acid, L-galactonic acid lactone, and D-glucuronic acid, which was consistent with *K. gwangyangensis* JCM 12864^T, while that of strain 5E331^T was negative as *K. lipolytica* JCM 30877^T (**Supplementary Table S1**). Strains A6E486^T and 5E331^T exhibited some similarities to *K. gwangyangensis* and *K. lipolytica* JCM 30877^T, including their abilities to hydrolyze Tweens (20, 40, and 60). The differential characteristics of strains A6E486^T and 5E331^T and experiment control strains were summarized in **Table 1** and **Supplementary Table S1**. Both strains A6E486^T and 5E331^T were susceptible to (μg per disc) penicillin (10), chloramphenicol (30), norfloxacin (30), cefotaxime sodium (30), gentamycin (10), ofloxacin (5), clarithromycin (15), carbenicillin (100), and ceftriaxone (30) but resistant to tetracycline (30) and kanamycin (30).

3.2 Chemotaxonomic Properties

The major cellular fatty acids (>10%) of strain A6E486^T consisted of iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{17:1}$ ω9c, and summed feature 3 (comprising $C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c) while those of strain 5E331^T were iso-$C_{15:0}$, iso-$C_{17:1}$ ω9c, and summed feature 3 (**Table 2**). The fatty acid compositions of the two strains were similar to experiment control strains but with differences in the proportions of some fatty acids. For example, iso-$C_{17:0}$ was one of the major fatty acids in strains A6E486^T and *K. gwangyangensis* JCM 12864^T but not in strains 5E331^T and *K. lipolytica* JCM 30877^T. Strains A6E486^T and 5E331^T had Q-10 as

TABLE 1 | Differential characteristics between strains A6E486^T and 5E331^T and experiment control strains.

Characteristic	1	2	3	4
Colony color	Beige	Beige and brown	Cream–white	Beige
Temperature range (°C)	20–43	15–40	17–44 [a]	15–45 [b]
NaCl range (%, w/v)	1.0–5.5	1.5–5.0	0.5–4.0 [a]	0.5–10 [b]
pH range	5.5–7.5	6.0–8.5	6.0–8.5 [a]	5.5–9.5 [b]
Voges–Proskauer reaction	+	+	+	–
Enzyme activity:				
Catalase	w	+	+	+
Gelatinase	–	+	–	+
Lipase (C14)	w	+	–	–
α-chymotrypsin	w	–	+	+
Hydrolysis of:				
Tween 80	w	+	+	++
Starch	–	+	+	–
Casein	+	+	+	–
Acid production from:				
Glycerol	–	–	w	+
L-xylose	+	w	–	–
D-glucose	+	–	+	+
D-fructose	+	+	–	+
D-mannose	–	–	+	+
Inositol	w	–	+	–
N-acetyl glucosamine	–	–	+	+
D-maltose	w	–	+	+
D-trehalose	+	–	+	–
L-rhamnose	–	–	–	+
Oxidation of:				
D-cellobiose	–	–	+	–
Sucrose	+	–	+	–
Glycerol	–	–	+	–
D-fructose-6-PO_4	+	w	–	–
Gelatin	+	–	+	–
L-histidine	+	w	+	–
α-keto-glutaric acid	+	+	+	–
Sodium butyrate	w	+	–	–

Strains: 1, A6E486^T; 2, 5E331^T; 3, K. gwangyangensis JCM 12864^T; 4, K. lipolytica JCM 30877^T. All data were from this study unless indicated otherwise. +, Positive; –, negative; w, weakly positive. All strains were positive for the following characteristics: alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, esculin ferric citrate, and potassium 5-ketogluconate.

Data from: a, Kwon et al. (2005); b, Wu et al. (2016).

TABLE 2 | Cellular fatty acid composition (%) of strains A6E486^T and 5E331^T and experiment control strains.

Fatty acid	1	2	3	4
Saturated				
$C_{12:0}$	TR	TR	2.0	1.0
$C_{16:0}$	6.4	4.1	5.3	3.2
$C_{17:0}$	1.9	TR	1.2	TR
Unsaturated				
$C_{15:1}$8c	1.7	1.4	–	1.8
$C_{15:1}$ w6c	TR	TR	–	1.0
$C_{17:1}$ w8c	3.7	3.1	1.4	4.5
$C_{17:1}$ w6c	6.5	4.5	1.2	6.8
Branched				
iso-$C_{15:1}$ F	1.2	2.1	TR	1.6
iso-$C_{15:0}$	**16.8**	**27.0**	**15.1**	**17.8**
iso-$C_{16:0}$	TR	1.5	TR	2.3
iso-$C_{17:0}$	**10.3**	2.5	**19.2**	3.2
iso-$C_{17:0}$ 3–OH	1.5	TR	1.02	TR
iso-$C_{17:1}$ w9c	**22.9**	**27.7**	**34.4**	**23.8**
Summed Feature				
3[a]	**16.2**	**17.3**	7.3	**23.2**
8[a]	3.9	1.5	5.1	TR

Strains: 1, A6E486^T; 2, 5E331^T; 3, K. gwangyangensis JCM 12864^T; 4, K. lipolytica JCM 30877^T. All data listed in the table are from this study. TR, trace (<1.0%); –, Not detected; Fatty acids present at >10% are indicated in bold.

[a]Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprises $C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c, and summed feature 8 comprises $C_{18:1}$ ω6c and/or $C_{18:1}$ ω7c.

the predominant respiratory quinone, which was consistent with the quinone type of the genus *Kordiimonas*. The major polar lipids detected in the two strains were phosphatidylethanolamine (PE), phosphatidylglycerol (PG), diphosphatidylglycerol (DPG), and unidentified glycolipids, aminolipids, and lipids. PE, PG, DPG, and unidentified glycolipids were detected in all tested *Kordiimonas* species (**Supplementary Figure S2**).

3.3 16S rRNA Gene Sequence and Phylogenetic Analysis

Nearly complete 16S rRNA gene sequences of strains A6E486^T (1,461 bp; GenBank accession number: MZ901372) and 5E331^T (1,462 bp; GenBank accession number: OM663707) were obtained according to the methods described in the *Materials and Methods* section. Comparative analysis results using EzBioCloud databases showed that strains A6E486^T and 5E331^T shared the highest 16S rRNA gene sequence similarity values of 96.8% and 96.1%, respectively, with *K. lipolytica* JCM 30877^T. NJ phylogenetic tree based on 16S rRNA gene indicated that strains A6E486^T and 5E331^T were affiliated with the genus *Kordiimonas* (**Figure 1**). Similar tree topologies were also obtained with the ML and MP algorithms.

3.4 Comparative Genomic Analysis of the Genus *Kordiimonas*

3.4.1 Comparison of Genomic Properties and Genetic Relatedness

The genome lengths of the seven species of the genus *Kordiimonas* (strains A6E486^T, 5E331^T, *K. gwangyangensis* JCM 30877^T, *K. lipolytica* JCM 12864^T, *K. lacus* CGMCC 1.9109^T, *K. pumila* N18^T, and *K. sediminis* KCTC 42590^T) were 3,243,597–4,557,767 bp with a mean size of 3,894,463 bp. The completeness values of these genomes were 99.6%–100.0%. Among them, strain 5E331^T had the minimum DNA G+C content (46.2%), while strain A6E486^T possessed the maximum content (59.9%). The NCBI PGAP results showed that a total of 3,436 genes were predicted for strain A6E486^T, including 3,381 protein-coding genes and 53 RNA genes (46 tRNA genes, 3 rRNA genes, and 4 ncRNA genes), while strain 5E331^T had 3,505 predicted genes. The detailed PGAP results of the seven species were shown in **Table 3**.

The POCP and ANI values were calculated to identify the genomic similarities of strains A6E486^T and 5E331^T with some members of the genus *Kordiimonas* (**Figure 2**). The POCP and ANI values between strain A6E486^T (strain 5E331^T) and some species of the genus *Kordiimonas* were 56.3%–67.3% (56.0%–61.5%) and 68.6%–73.1% (68.8%–69.9%), respectively. The POCP values were higher than the genus delineation threshold (50%) (Qin et al., 2014a), and the ANI values were lower than the species delineation threshold (95%) (Yoon et al., 2017), which indicated that strains A6E486^T and 5E331^T represented two novel species of the genus *Kordiimonas*. The ribosomal protein phylogenetic tree showed the evolutionary relationships of some members of genus *Kordiimonas* (**Figure 2**).

3.4.2 Pan-Genome Analysis of the Genus *Kordiimonas*

Pan-genome analysis using BPGA was performed to identify orthologous groups among strains A6E486^T, 5E331^T, *K. gwangyangensis* JCM 30877^T, *K. lipolytica* JCM 12864^T, *K. lacus* CGMCC 1.9109^T, *K. pumila* N18^T, and *K. sediminis* KCTC 42590^T. Comparative analysis based on orthologous groups of proteins revealed that 1,258 core genes were shared by the seven species (**Figure 3A**). The percentages of core genes

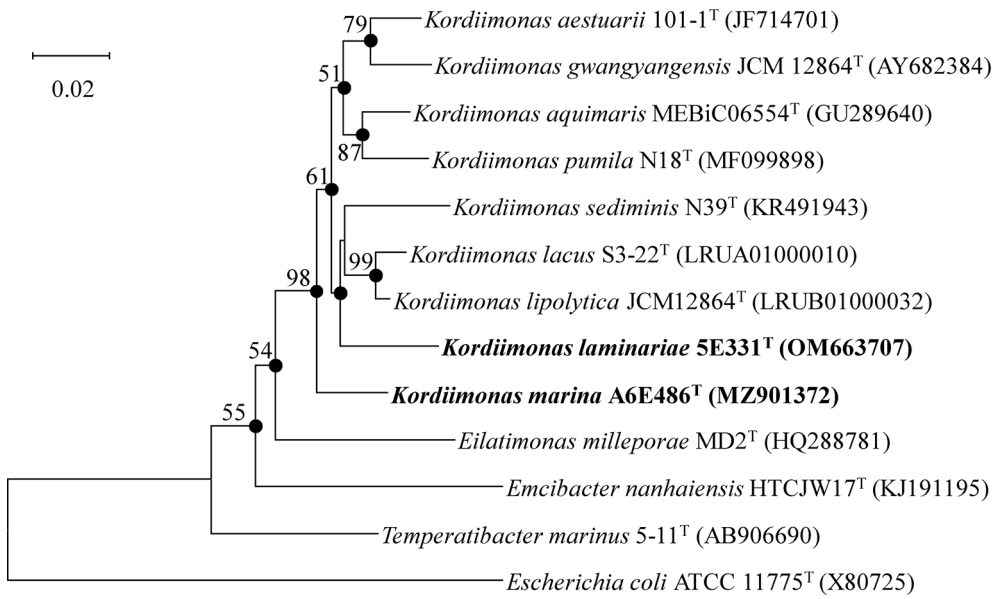

FIGURE 1 | Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strains A6E486^T and 5E331^T and other closely related species. Filled circles indicate branches that were recovered with all three methods (neighbor-joining, maximum-likelihood, and maximum-parsimony). Percentage bootstrap values above 50% (1,000 replicates) are shown at branch nodes. *Escherichia coli* ATCC 11775^T was used as the out-group. Bar, 0.02 substitutions per nucleotide position.

in each genome ranged from 34.3% to 44.1% (**Figure 3B**), and compared with unique genes and accessory genes, core genes contributed more to energy metabolism, nucleotide metabolism, replication and repair, and translation (**Supplementary Figure S3**). The proportion of accessory genes and unique genes in the seven genomes varied greatly (**Figure 3B**). Accessory genes and unique genes were more distributed than core genes in amino acid metabolism, carbohydrate metabolism, signal transduction, and xenobiotic biodegradation and metabolism (**Supplementary Figure S3**), which indicated that these genes conferred metabolic diversity and marine environment adaptability to the members of the genus *Kordiimonas* (Innamorati et al., 2020). Results of the core pan-genome prediction model showed that the pan-genome increased with the addition of new strains and was far from saturation (**Supplementary Figure S4**), which indicated that the pan-genome of the genus *Kordiimonas* was open. Compared with a previous study (Geng et al., 2022), the complement of strains A6E486^T and 5E331^T in this study reduced the number of shared core genes of the genus *Kordiimonas*, showing that core genes decreased with the increase of species (**Supplementary Figure S4**).

3.4.3 Metabolic Pathway Analysis

Metabolic pathways were analyzed using KEGG's BlastKOALA service. The results showed that the seven species of the genus *Kordiimonas* had the most genes in gene information processing, followed by signaling and cellular processes and carbohydrate metabolism (**Supplementary Figure S5**). In addition, the metabolic pathways of the seven species were compared and analyzed, including carbohydrate metabolism, energy

TABLE 3 | Genome statistics of strains A6E486^T and 5E331^T and five members of the genus *Kordiimonas*.

	1	2	3	4	5	6	7
Genome size (bp)	3,684,098	3,651,059	4,082,245	4,557,767	4,000,817	4,041,658	3,243,597
Completeness (%)	99.9	100.0	100.0	100.0	99.6	100.0	100.0
Contigs	25	27	13	36	12	1	12
N_{50} length (bp)	603,855	474,336	686,379	229,908	482,103	4,041,658	1,727,258
G+C content (mol %)	59.9	46.2	57.5	56.3	57.2	47.4	49.6
Genes	3,436	3,505	3,727	4,286	3,688	3,608	2,908
Protein-coding genes	3,381	3,434	3,667	4,169	3,623	3,517	2,842
tRNA genes	46	47	43	51	42	42	43
rRNA genes	3	3	5	9	5	6	5
ncRNA genes	4	3	4	4	3	4	4
GenBank ID	JAINDF 000000000	JAKQZA 000000000	AQXF 01000000	LRUB 01000000	FNAK 01000000	CP061205	BNCI 01000000

Strains: 1, A6E486^T; 2, 5E331^T; 3, K. gwangyangensis JCM 30877^T; 4, K. lipolytica JCM 12864^T; 5, K. lacus CGMCC 1.9109^T; 6, K. pumila N18^T; 7, K. sediminis KCTC 42590^T.

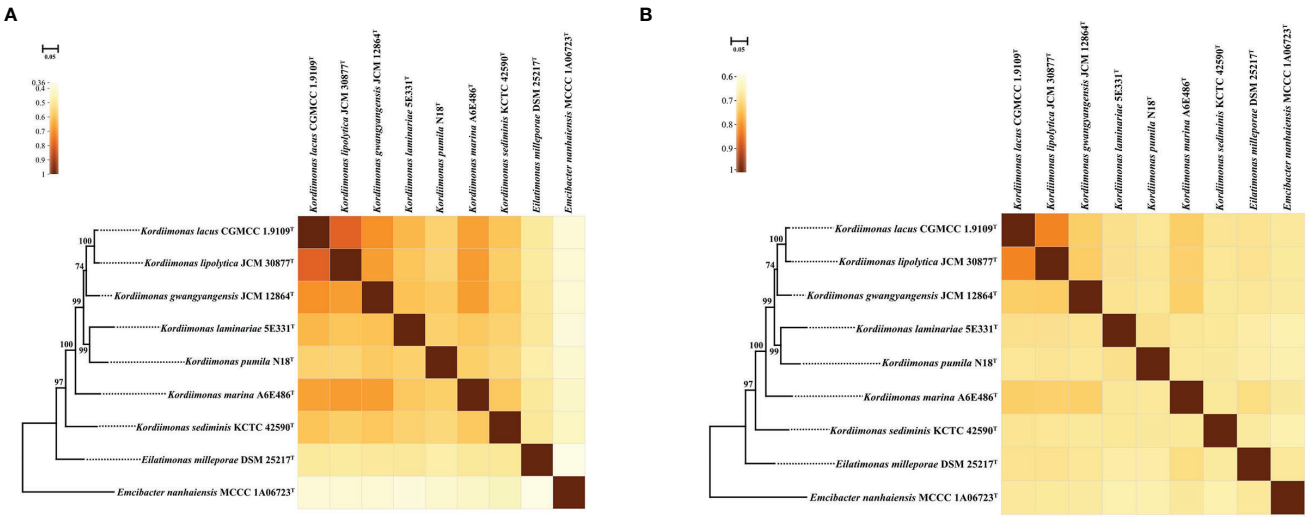

FIGURE 2 | Heat maps showing POCP and ANI values among A6E486^T, 5E331^T, and members of the family *Temperatibacteraceae*. **(A)** Heat map based on POCP values. **(B)** Heat map based on ANI values. IQ-TREE based on ribosomal protein sequences shows the phylogenetic relationships among these strains. Percentage bootstrap values (1,000 replicates) are shown at branch nodes. *Emcibacter nanhaiensis* MCCC 1A06723^T was used as the out-group. Bar, 0.05 substitutions per nucleotide position. Same tree topology was also obtained using FastTree. ANI, average nucleotide identity; POCP, percentage of conserved proteins.

metabolism, lipid metabolism, amino acid metabolism, and metabolism of cofactors and vitamins. Among the seven *Kordiimonas* species, all strains have complete pathways of gluconeogenesis (M00003), pyruvate oxidation (M00307), citrate cycle (M00009), pentose phosphate pathway (M00007), and glyoxylate cycle (M00012), whereas only strains A6E486^T and *K. lipolytica* JCM 30877^T possess a complete glycolysis pathway (M00001) (**Figure 4**). The seven species also have similarities and differences in other metabolic pathways. For example, a complete PE biosynthesis pathway (M00093) was found in all strains, which was consistent with the polar lipid results of the genus *Kordiimonas*. The initiation pathways of fatty acid synthesis (M00082) and cobalamin biosynthesis (M00122) are complete only in strain 5E331^T, while the cytochrome o ubiquinol oxidase (M00417) and betaine biosynthesis (M00555) pathways are complete only in strain A6E486^T (**Figure 4**). Considering that the incomplete glycolysis pathway in most *Kordiimonas* strains leads to pyruvate deficiency and thus to the decrease of acetyl-CoA from the pyruvate oxidation pathway, the leucine degradation pathway is important due to the production of acetyl-CoA (**Figure 5A**). It was proven that strains A6E486^T, 5E331^T, *K. gwangyangensis* JCM 30877^T, and *K. lipolytica* JCM 12864^T could grow slowly in the medium with leucine as the solo carbon source (**Supplementary Figure S6**). In addition, dTDP-L-rhamnose biosynthesis pathway (M00793) was completely annotated in the genomes of the seven *Kordiimonas* species (**Figure 5B**).

3.4.4 Prediction of Secondary Metabolites

AntiSMASH server was used to predict the secondary metabolites of the seven species. Results showed that all of the seven species contained gene clusters encoding RiPP-like (Other unspecified ribosomally synthesized and posttranslationally modified peptide product). Except for *K. gwangyangensis* JCM 12864^T and *K. pumila* N18^T, the genomes of the remaining five strains contained terpene gene clusters. The gene clusters encoding hserlactone (homoserine lactone) were found in genomes of the six strains except *K. pumila* N18^T, which had β-lactone (β-lactone containing protease inhibitor) gene clusters (**Supplementary Table S2**). Homoserine lactones, as one of the most important signal molecules in the quorum-sensing system of Gram-negative bacteria, regulate the expression of many physiological characteristics (Parsek and Greenberg, 2000; Qin et al., 2020). Cluster Pfam (Protein Families Database) analysis and Pfam-based GO (Gene Ontology) term annotation results showed that the core biosynthetic genes in all of the hserlactone gene clusters encoded autoinducer synthase, and there were additional biosynthetic genes encoding luxR family regulatory proteins upstream and downstream of the core genes (**Supplementary Figure S7**). The luxR regulators are important components of the quorum-sensing system, which are distributed to sense and respond to N-acyl homoserine lactone by autoinducer-binding domain (Subramoni and Venturi, 2009; Santos et al., 2012).

3.4.5 Comparative Analysis of Carbohydrate-Active Enzymes

Considering that the strains had differences in acid production and oxidation with different carbohydrates as substrates (**Table 1**; **Supplementary Table S1**), carbohydrate-active enzymes of the genus *Kordiimonas* were annotated and analyzed. The results showed that *K. sediminis* KCTC 42590^T had the least carbohydrate-active enzymes (86), while *K. gwangyangensis* JCM 30877^T had the most (137). Among

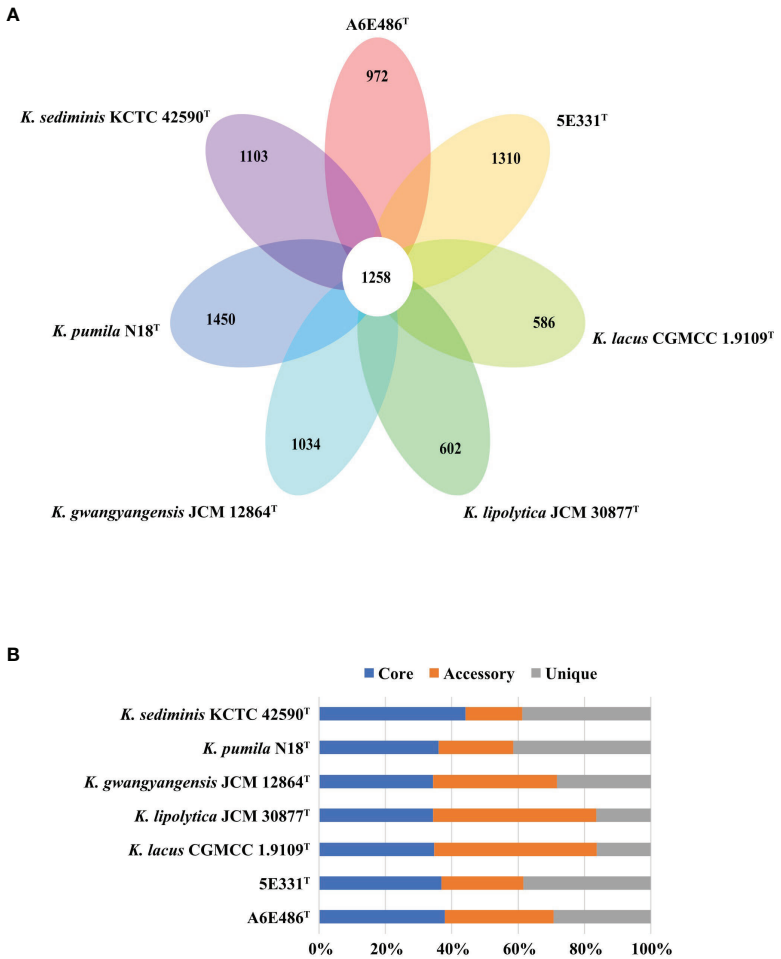

FIGURE 3 | Comparisons of *Kordiimonas* orthologous protein groups in the seven *Kordiimonas* genomes. **(A)** Venn diagram displaying the numbers of core gene families and unique genes for each of the seven *Kordiimonas* strains. **(B)** Percentage of core, accessory, and unique genes in each of the seven genomes.

these carbohydrate-active enzymes, most enzymes in the genomes of the seven species were assigned to the glycosyltransferase (GT) family and glycoside hydrolase (GH) family, which was consistent with a previous study (Geng et al., 2022); however, polysaccharide lyases (PLs) were found in parts of the *Kordiimonas* species (**Figure 6**). Strain A6E486^T had 53 GTs and 50 GHs, which were more than those in strain 5E331^T, *K. lipolytica* JCM 12864^T, *K. lacus* CGMCC 1.9109^T, *K. pumila* N18^T, and *K. sediminis* KCTC 42590^T. Strain 5E331^T harbored 107 carbohydrate-active enzymes, including 42 GTs, 36 GHs, 16 carbohydrate esterases (CEs), 6 carbohydrate-binding modules (CBMs), and 7 auxiliary activities (AAs). Detailed comparison results were shown in **Figure 6**.

3.4.6 Analysis of Marine Environment Adaptability

Considering that members of the genus *Kordiimonas* were all isolated from marine environments and habitats, metabolic features related to functional categories were analyzed to investigate the marine-adapted lifestyle of the genus *Kordiimonas* (**Table 4**). In the first place, 26–51 genes associated with "Virulence, Disease and Defense" were found in the genomes of members of the genus *Kordiimonas*, which may be crucial for bacteria to resist marine environment pollutants including heavy-metal ions, antibiotics, and other toxic compounds. Secondly, flagellar motility and bacterial chemotaxis genes were found, and there were differences in the number of the related genes. Most of the marine environments are oligotrophic; flagellar motility and bacterial chemotaxis genes, which assist marine bacteria in the acquisition of nutrients, may play an important role in the marine sediment-adapted lifestyle (Qin et al., 2011). Thirdly, there were different numbers of genes related to capsular and extracellular polysaccharides and polysaccharide export proteins in the genomes. Exopolysaccharides (EPSs) are essential for the survival of bacteria in marine environments, as EPSs assist them to endure extremes of temperature, salinity, and nutrient availability (Nichols et al., 2005). The existence of genes related to EPS synthesis and export suggested that members of the genus

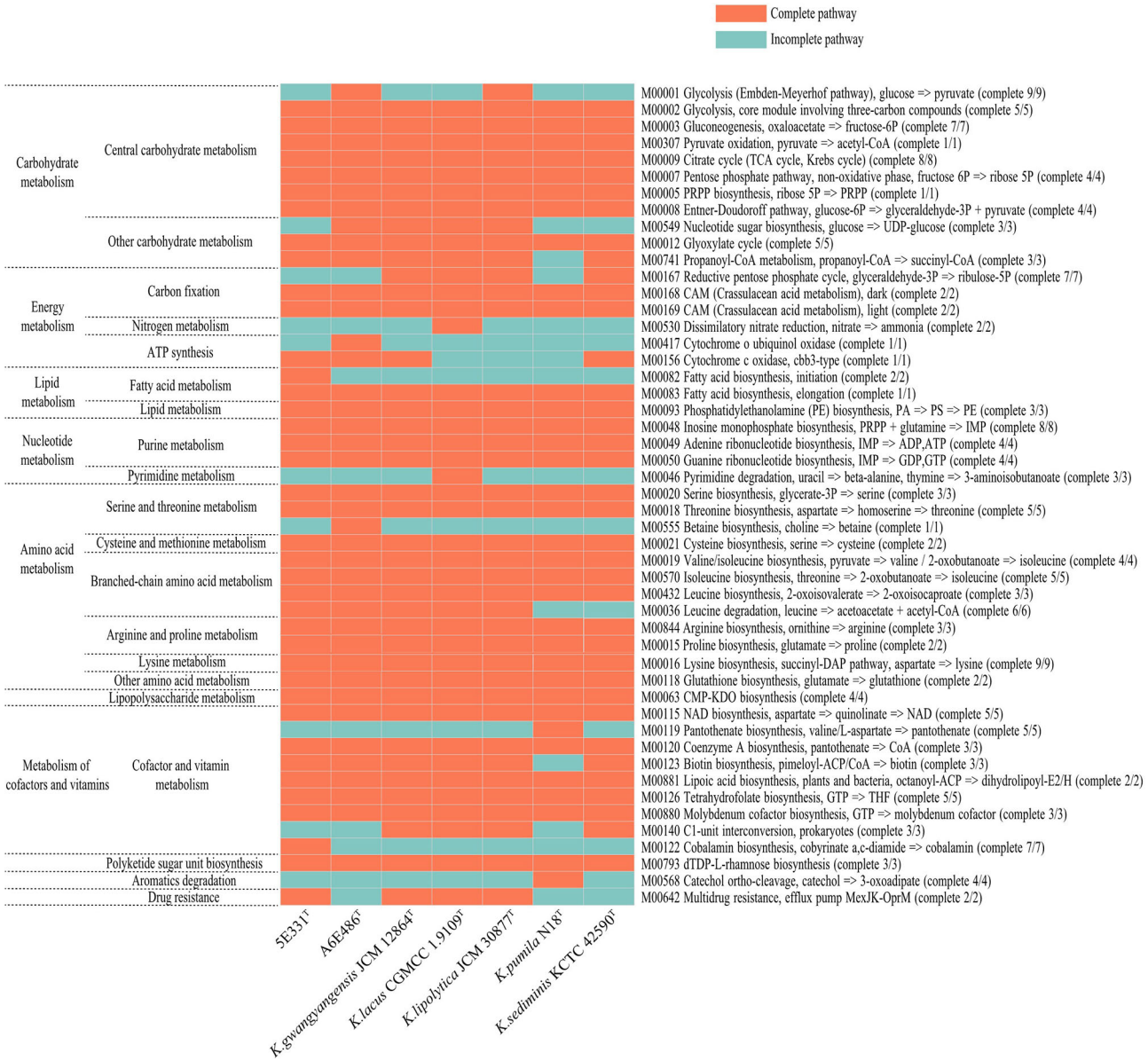

FIGURE 4 | Heat maps of complete and incomplete metabolic pathways in the genomes of strains A6E486^T, 5E331^T, *K. gwangyangensis* JCM 30877^T, *K. lipolytica* JCM 12864^T, *K. lacus* CGMCC 1.9109^T, *K. pumila* N18^T, and *K. sediminis* KCTC 42590^T.

Kordiimonas may be able to resist low temperature through EPS secretion. Additionally, cold-shock proteins and tRNA-dihydrouridine synthase play roles in the cold-adapted lifestyle of marine bacteria by helping protein folding and increasing tRNA flexibility at low temperatures, respectively (Qin et al., 2014b). Fourthly, all members of the genus *Kordiimonas* had numerous genes involved in the "stress response," which may give these species the ability to cope with pressures such as oxygen and temperature in marine environments.

Considering the high osmotic pressure of marine environments and the protective effect of compatible solutes on microorganisms under high osmotic condition (Da et al., 1998; Wood et al., 2001), compatible solute synthesis and transport of the genus *Kordiimonas* were analyzed using the KEGG database. The results showed that only strain A4E486^T had a complete betaine synthesis pathway (M00555) among the seven species of the genus *Kordiimonas* (**Figure 4**). Strains 5E331^T, *K. gwangyangensis* JCM 30877^T, *K. lipolytica* JCM 12864^T, *K. lacus* CGMCC 1.9109^T, *K. pumila* N18^T, and *K. sediminis* KCTC 42590^T encode choline dehydrogenase (CHDH), which converts choline to betaine aldehyde (Zou et al., 2016). The genes encoding glycine betaine transporter were found only in the genome of strain A4E486^T. In

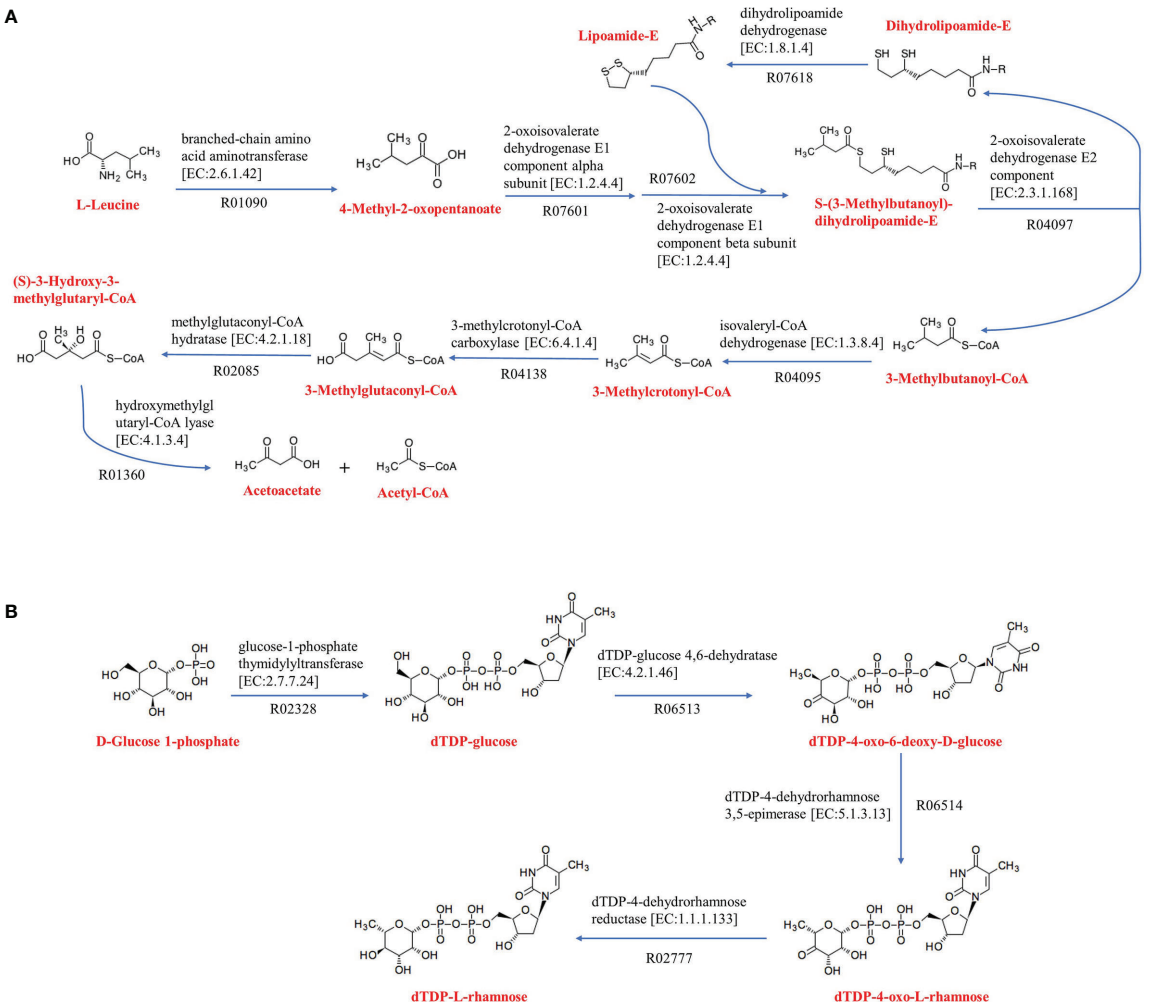

FIGURE 5 | Amino acid metabolism and polyketide sugar unit biosynthesis of *Kordiimonas* strains. **(A)** Leucine degradation. **(B)** dTDP-L-rhamnose biosynthesis. The metabolic pathway maps are based on the KEGG analysis. Other reactants and products have been omitted.

the hypertonic environment, the accumulation of amino acids in cells, such as proline (Hoffmann et al., 2012) and arginine (Xu S. et al., 2011), also plays a positive role in the anti-osmotic ability of microorganisms (Da et al., 1998; Roesser and Muller, 2001). The seven genomes had the proline biosynthesis pathway (M00015) and arginine biosynthesis pathway (M00844) identified by the KEGG database (**Figure 4**). Numerous complete amino acid synthesis pathways in members of the genus *Kordiimonas* may help to resist cell damage caused by high osmotic pressure in marine environments. Finally, the antioxidant systems used to scavenge free radicals were analyzed. The antioxidant system mainly includes superoxide dismutase, catalase, glutathione, and cytochrome oxidase (Pomposiello and Demple, 2002). The analysis showed that all seven species of the genus *Kordiimonas* encoded superoxide dismutase (Fe-Mn family) and catalase-peroxidase. Strains 5E331^T and *K. pumila* N18^T had genes that encoded catalase. The genes encoding cytochrome c peroxidase were found in strains 5E331^T, *K. gwangyangensis* JCM 30877^T, *K. lipolytica* JCM 12864^T, *K. lacus* CGMCC 1.9109^T, and *K. sediminis* KCTC 42590^T. Additionally, all seven members of the genus *Kordiimonas* had a complete glutathione synthesis pathway (M00118) (**Figure 4**).

The analysis of metabolic features and related genes revealed the genetic basis of the genus *Kordiimonas* to adapt to the marine environment.

4 DESCRIPTION OF *KORDIIMONAS MARINA* SP. NOV.

Kordiimonas marina (ma.ri'na. L. fem. adj. marina, pertaining to the isolation of the type strain from the marine environment).

Cells are Gram-negative and facultatively anaerobic with rod shape. Growth is observed at pH 5.5–7.5 (optimum, 6.5) and temperatures of 20°C–43°C (optimum, 33°C–35°C) and with 1.0%–5.5% (w/v, optimum, 2.5%–3.0%) NaCl. Growth occurs

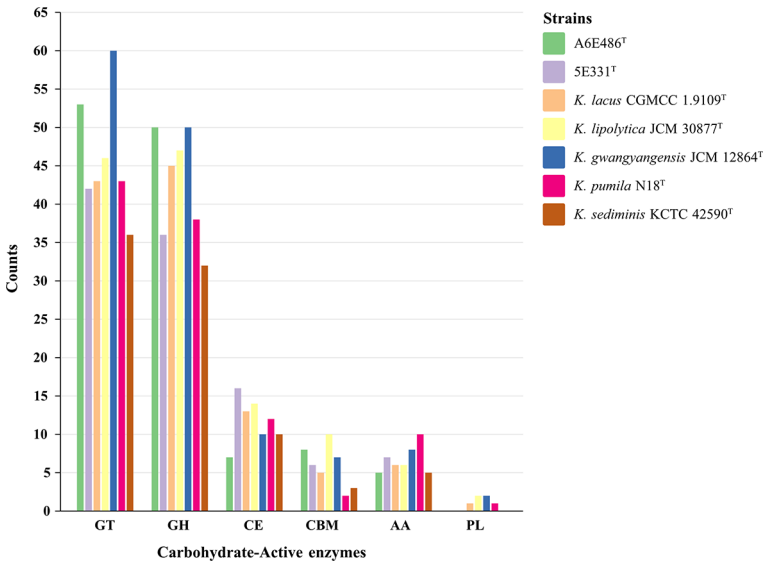

FIGURE 6 | Histogram of predicted carbohydrate-active enzymes in strains A6E486^T, 5E331^T, *K. gwangyangensis* JCM 30877^T, *K. lipolytica* JCM 12864^T, *K. lacus* CGMCC 1.9109^T, *K. pumila* N18^T, and *K. sediminis* KCTC 42590^T. Numbers of each enzyme detected in the genomes were shown in the map. GT, glycosyltransferase; GH, glycoside hydrolase; CE, carbohydrate esterase; CBM, carbohydrate-binding module; AA, auxiliary activity; PL, polysaccharide lyase.

under anaerobic conditions, and nitrate is reduced to nitrite. The activities of trypsin and oxidase are positive, and the activities of α-chymotrypsin, α-galactosidase, α-glucosidase, α-mannosidase, and α-fucosidase are weakly positive. Acids are produced from D-arabinose, D-ribose, D-xylose, L-sorbose, D-tagatose, and potassium 5-ketogluconate. The major cellular fatty acids (>10%) are iso-$C_{15:0}$, iso-$C_{17:0}$, iso-$C_{17:1}$ ω9c, and summed feature 3 (comprising $C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c). The predominant respiratory quinone is Q-10. The major polar lipids consist of PE, PG, DPG, and unidentified glycolipids, aminolipids, and lipids. The DNA G+C content of type strain is 59.9 mol%.

The type strain is A6E486^T (= KCTC 82758^T = MCCC 1H00470^T), which was isolated from coastal sediments collected off the coast of Weihai, China.

5 DESCRIPTION OF *KORDIIMONAS LAMINARIAE* SP. NOV.

Kordiimonas laminariae (la.mi.na'ri.ae. N.L. gen. fem. n. laminariae, pertaining to the kelp Laminaria, from which the type strain was isolated).

TABLE 4 | Metabolic features and selected genes related to the adaptation to marine environments.

Categories	Metabolic features	Gene Count						
		1	2	3	4	5	6	7
Virulence, disease, and defense	*Mycobacterium* virulence operon	18	16	15	15	15	13	14
	Copper resistance	9	9	8	12	9	9	7
	Cobalt-zinc-cadmium resistance	4	10	4	11	7	8	2
	Fluoroquinolones resistance	2	2	2	2	2	2	2
	β-lactamase	1	2	1	2	2	1	1
Motility and chemotaxis	Flagellar motility	65	33	70	84	72	47	24
	Bacterial chemotaxis	17	22	26	30	19	21	15
Cold adaptation	Capsular and extracellular polysaccharides	14	17	28	18	20	27	11
	Polysaccharide export proteins	4	5	2	5	5	2	3
	Cold-shock proteins	4	3	3	4	1	3	1
	tRNA-dihydrouridine synthase	1	1	1	1	1	1	1
Stress response	Osmotic stress	4	0	1	2	2	2	1
	Oxidative stress	33	36	43	41	38	30	29
	Detoxification	9	7	8	7	6	6	7
	Phage shock protein operons	4	5	4	8	4	4	4
	Heat-shock proteins	7	5	6	5	7	5	2

Strains: 1, A6E486^T; 2, 5E331^T; 3, K. gwangyangensis JCM 30877^T; 4, K. lipolytica JCM 12864^T; 5, K. lacus CGMCC 1.9109^T; 6, K. pumila N18^T; 7, K. sediminis KCTC 42590^T.

Cells are Gram-negative and aerobic with rod shape. Growth is observed at pH 6.0–8.5 (optimum,7.0) and temperatures of 15°C–40°C (optimum, 33°C) and with 1.5%–5.0% (w/v, optimum, 3.0%) NaCl. Growth does not occur under anaerobic condition, and nitrate is not reduced to nitrite. The activities of trypsin and oxidase are positive, but the activities of α-chymotrypsin, α-galactosidase, α-glucosidase, α-mannosidase, and α-fucosidase are negative. Acids are produced from D-ribose, L-sorbose, D-turanose, D-lyxose, D-tagatose, and potassium 5-ketogluconate. The major cellular fatty acids (>10%) are iso-$C_{15:0}$, iso-$C_{17:1}$ ω9c, and summed feature 3 (comprising $C_{16:1}$ ω6c and/or $C_{16:1}$ ω7c). The predominant respiratory quinone is Q-10. The major polar lipids consist of PE, PG, DPG, and unidentified glycolipids, aminolipids, and lipids. The DNA G+C content of type strain is 46.2 mol%.

The type strain is 5E331^T (= KCTC 92199^T = MCCC 1H00515^T), which was isolated from fresh kelps collected from a kelp culture area, Rongcheng, China.

DATA AVAILABILITY STATEMENT

The data presented in this study are deposited in the GenBank. The accession number for the 16S rRNA gene sequence of strains A6E486^T and 5E331^T are MZ901372 and OM663707, respectively. The accession number for the whole genome shotgun project of strains A6E486^T and 5E331^T are JAINDF000000000 and JAKQZA000000000, respectively.

AUTHOR CONTRIBUTIONS

Y-QY and Z-PH isolated the strain A6E486^T and performed material preparation, experimental operation, and data collection and analysis. Y-YY helped process cell samples and photograph cell morphology. LM isolated the strain 5E331^T, and Y-QY finished the experiment and article. Z-JD and M-QY offered experiment guidance and critical revision of the article. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.919253/full#supplementary-material

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