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Chondrinema litorale gen. nov., sp. nov., of the phylum *Bacteroidota*, carrying multiple megaplasmids isolated from a tidal flat in the West Sea, Korea

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A Gram-stain-negative, long rod, oxidase and catalase-positive strain WSW3-B12^T was isolated from red algae on tidal flats in the West Sea, Korea. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that the strain WSW3-B12^T had the highest sequence similarity, 92.7%, to *Flexithrix dorotheae* DSM 6795^T, followed by *Rapidithrix thailandica* TISTR 1750^T at 90.8% in the family *Flammeovirgaceae* of the phylum *Bacteroidota*. The whole genome sequence determined using both the Nanopore and Illumina platforms revealed that the complete genome consists of 29 contigs, among which contig 1 was a circular chromosome, while the remaining 28 contigs were plasmids. The size of the genome was 10.1 Mbp and the G+C content was 34.1%. The average nucleotide identity (ANI), digital DNA–DNA hybridization (dDDH), average amino acid identity (AAI), and percentage of conserved proteins (POCP), phylogenomic-related indexes between the strain WSW3-B12^T and the closest strain *Flexithrix dorotheae* DSM 6795^T, were 76.6%, 19.9%, 57.2%, and 55.6%, respectively, which were all lower than the threshold values to support the creation of a novel genus. A comprehensive genome analysis revealed that the strain WSW3-B12^T harbored many of the key genes involved in central metabolism in the main chromosome and also carried important genes for the production of vitamins, quinone, and antimicrobial resistance on the plasmids. The strain also carried genes that are involved in the metabolism of heavy metals such as arsenic, cobalt, copper, and iron on both the chromosome and plasmids. Furthermore, the genome of the strain was highly enriched with carbohydrate-active enzymes (CAZymes), carrying a total of 241 CAZymes. Moreover, a complete CRISPR/Cas system was detected on plasmid 20. The major fatty acids of the strain were iso-$C_{15:0}$ and $C_{16:1}$ ω5. The polar lipids contained phosphatidylethanolamine, four unidentified lipids, and four glycolipids. The respiratory quinone was menaquinone 7. Based on the phenotypic,

chemotaxonomic, and genomic analyses, the strain WSW3-B12^T could be assigned to a novel species and novel genus within the family *Flammeovirgaceae*, for which the name *Chondrinema litorale* gen. nov., sp. nov. (type strain WSW3-B12^T = KCTC 82707^T = GDMCC 1.3198^T) is proposed.

KEYWORDS

red algae, microbial diversity, polyphasic taxonomy, comparative genomics, carbohydrate-active enzymes

1 Introduction

The sea covers around 70% of the surface of our planet, and tidal flats connect the sea and land. Tidal flats are located between marine ecosystems and land environments, providing a habitat for aquatic organisms and an ideal place for isolating unique microbial taxa (Wilms et al., 2006). They are exposed to certain environmental events, such as flooding, habitat destruction, climate changes, and pollution (Duarte et al., 2008), which result in the formation of unique microbial communities (Lv et al., 2016). Microbes in tidal flats play critical roles in regulating ecological processes and biogeochemical cycles (Beam et al., 2020). They are a key player in nutrient cycling by converting organic matter into inorganic forms, which enhance the nutrient supply in marine environments (Lee et al., 2021). Furthermore, these microbes also decompose pollutants flowing into the tidal flats (Cheng et al., 2017; Lee et al., 2019; Kim et al., 2022). The productivity of tidal flat ecosystems is largely due to these microbes. However, the precise relationship between their diversity and functioning is little understood.

The microbes in the tidal flats interact with invertebrates, animals, plants, and seaweed. Seaweed or sea macroalgae, which perform primary production using sunlight and nutrients from the environment, refer to thousands of species of macroscopic, multicellular marine algae. Macroalgae include red, brown, and green algae, which are essential components of the marine food chain (Lobban and Harrison, 1994). Furthermore, macroalgae are biological reservoirs of abundant marine bacteria (Singh and Reddy, 2015; Juhmani et al., 2020). These microbes produce bioactive compounds that can be used to combat drug resistance and cancer (Menaa et al., 2020; Kizhakkekalam and Chakraborty, 2021). A large number of novel bacterial strains have been isolated from marine algae (Karthick and Mohanraju, 2018; Asharaf et al., 2022; Muhammad et al., 2022a).

The phylum *Bacteroidota* (a heterotypic synonym of *Bacteroidetes*) was first established by Leadbetter in 1974 (Leadbetter, 1974), subsequently amended by Reichenbach in 1992 (Reichenbach, 1992), and again revised by Ludwig (Ludwig et al., 2010). The phylum was initially described as a monophyletic branch of three genera, *Cytophaga, Flavobacterium*, and *Bacteroides* (CFB) (Paster et al., 1994). But it was expanded to include six classes and six orders (https://lpsn.dsmz.de/phylum/bacteroidota). The class *Cytophagia* is currently represented by the sole order *Cytophagales* (https://lpsn.dsmz.de/order/cytophagales) comprising 20 families, among which *Flammeovirgaceae* (https://lpsn.dsmz.de/family/flammeovirgaceae) is the second largest family, comprising nine validly published genera and three genera unassigned to the family. The nine validly published genera are *Flammeovirga, Flexithrix, Imperialibacter, Limibacter, Perexilibacter, Rapidithrix, Sediminitox, Tunicatimonas*, and *Xanthovirga*.

Members of the family *Flammeovirgaceae* are heterotrophic, Gram-stain-negative, non-flagellated, non-spore-forming, rod-shaped cells, and share menaquinone 7 (MK-7) as the major respiratory quinone (Yoon et al., 2011). They are widely distributed in various environments, particularly in marine aquatic systems (Yoon et al., 2012; Wang et al., 2013; Goldberg et al., 2020). Members of the family *Flammeovirgaceae* and other members of the phylum *Bacteroidota* are relatively enriched in a large number of glycoside hydrolases (GHs), glycosyl transferases, peptidases, and adhesion proteins that degrade organic biomacromolecules (Fernández-Gómez et al., 2013). They contain a large number of genes encoding carbohydrate-active enzymes, which potentially participate in polysaccharide degradation (Mckee et al., 2021). There are a number of reported strains of the phylum *Bacteroidota* that can degrade various types of complex polysaccharides (Hehemann et al., 2012; Mann et al., 2013).

During a study on the microbial diversity of the tidal flats in the West Sea, Korea, a number of novel bacterial strains were isolated (Muhammad et al., 2022a; Muhammad et al., 2022b). Strain WSW3-B12^T isolated from the red algae of the genus *Chondrus* was characterized by polyphasic taxonomy and genome analysis. Based on the polyphasic taxonomic analysis, it is proposed that the isolate represents a novel species and a novel genus of the family *Flammeovirgaceae* in the phylum *Bacteroidota*. A comparative genomic analysis of the strain WSW3-B12^T and its close strains highlighted the uniqueness in terms of multiple plasmids and the presence of various functional genes for possible application. Genome mining emphasized the presence of abundant genes for heavy metal metabolism, for CAZymes of polysaccharide degradation, and for CRISPR candidates that are expected to have potential biotechnological applications. Furthermore, the degradation of polysaccharides (starch, inulin, and cellulose) and the presence of heavy metal metabolism genes (arsenic, cobalt, and

copper) highlighted the contribution of strain in the carbon cycle (Fernández-Gómez et al., 2013) and its important role in the remediation of heavy metals (Chen et al., 2018; Dell'Anno et al., 2021) in the tidal flat ecosystem.

2 Materials and methods

2.1 Isolation and cultivation

A red alga belonging to the genus *Chondrus* was collected in the West Sea, Byeonsan, Republic of Korea (35° 40′ 53.76″ N 126° 31′ 51.96″ E) in late autumn, 2020. The red alga genus *Chondrus* is abundant across the east and west coasts of the Republic of Korea. The genus can be found on rocks from the middle intertidal zone into the subtidal zone (Lee et al., 2013; Kristyanto et al., 2022). One gram of the alga was cut into small pieces and then placed at the center of agar plates, which was composed of 60% seawater and 1.5% agar. The primary plates were incubated at 15 °C for five days and then routinely observed under a stereoscopic microscope. The colonies from the primary plate were picked up and transferred to marine agar 2216 (MA; BD) at 30°C which is also called Zobell media, a standard plate medium for the cultivation of marine bacteria (ZoBell, 1941). The pure cultures of the novel strain were preserved in 20% glycerol at -80°C. The cultures were deposited in the Korean Collection for Type Cultures (KCTC) and the Guangdong Microbial Culture Collection Center (GDMCC), under the culture depositing numbers KCTC 82707^T and GDMCC 1.3198^T.

2.2 16S rRNA phylogeny

The genomic DNA of the strain WSW3-B12^T was extracted from cells grown in MA at 30°C, by a NucleoSpin Microbial DNA kit (Macherey-nagel, Germany) following the manufacturer's instructions. The 16S rRNA gene was amplified with bacterial universal primers and sequenced with Sanger's sequencing method (Biofact, South Korea) (Pheng et al., 2020). Pairwise sequence similarity values of the 16S rRNA gene sequence (1454 bp) were obtained through the EzBioCloud server (http://www.ezbiocloud.net) (Yoon et al., 2017a). All sequences of the related strains retrieved from EzBioCloud were aligned and edited with the BioEdit software (version 7.2.5). Phylogenetic trees were constructed by using neighbor-joining (NJ) (Saitou and Nei, 1987), maximum-likelihood algorithms (ML) (Felsenstein, 1981), and the maximum parsimony (MP) method (Felsenstein, 1981) in the Molecular Evolutionary Genetics Analysis (MEGA X) program (Kumar et al., 2018). For the ML tree, Kimura's two-parameter model was used for the calculation of the evolutionary distance. For the construction of NJ and MP phylogenetic trees Tajima-Nei model and subtree-pruning-regrafting (SRF) model were used. The bootstrap resampling method of 1000 replicates was applied to evaluate the phylogenetic tree. Bootstrap values >70% are presented in nodes of each branch.

2.3 Morphological, physiological, and biochemical analyses

Gram-staining was performed with the BBL^{TM} Gram-staining kit (BD, USA) according to the manufacturer's instructions. Cells of the strain WSW3-B12^T were incubated for 48 h on MA at 30 °C and examined for morphology through a scanning electron microscope (Regulus 8100, Hitachi) (Jeon et al., 2022). The motility was tested by the hanging drop method (Bowman, 2000). The optimal temperature for growth was determined in MA, for seven days at 4, 10, 15, 20, 25, 30, 37, and 45 °C. The salt tolerance was determined for a novel and reference strains by making a media having the same composition as MA except with the supplementation of salt from 0 to 0.5, and 1.0-8.0% (w/v) (Muhammad et al., 2022a). Growth at pH 5 to 10 (at intervals of 0.5 unit) was determined in MA adjusted with various buffers, at a concentration of 20 mM: acetate for pH 5.0–5.5, phosphate for pH 6.0–8.0, Tris for pH 8.5–9.0, and carbonate for pH 9.5–10.0 (Muhammad et al., 2022b). Catalase activity was tested by the production of oxygen bubbles when 3% (v/v) H_2O_2 was added to fresh cells (Ueno et al., 2021). The oxidase reaction was carried out using 1% (w/v) tetramethyl-*p*-phenylenediamine.

The degradation of polysaccharides was studied in basal media made of 60% (v/v) seawater, 0.1% (w/v) peptone, 0.6% (w/v) gellan gum as a solidifying agent, and 0.1% (w/v) of each test polysaccharide (cellulose, chitin, κ-carrageenan, inulin, sodium alginate, starch, and xylan) (Gao et al., 2017). The degradation of polysaccharides was further determined by using basal broth, which contained 0.2% of the above sugar molecules. Each sugar broth was inoculated with a 48 hours' old culture and incubated at 30 °C in the shaker. The supernatant of the culture was harvested at 0, 2, and 4 days and then reacted with 3,5-dinitrosalicylic acid (DNS) to detect reduced sugar production (Gao et al., 2017; Deshavath et al., 2020). The hydrolysis of Tweens 20, 40, 80, and casein was determined by the method of Cowan & Steel (Phillips, 1993; Amrina et al., 2021). DNase activity was checked by using DNase agar (BD).

The enzymatic activities and carbon source utilization were determined by using API ZYM, API 20E, API 50CH (bioMérieux), and GEN III Microplates (Biolog), following the manufacturer's instructions, except that the inoculum suspensions were adjusted to a final concentration of 2% (w/v) NaCl (Jeong et al., 2020). To test for growth under an anaerobic condition, the strain WSW3-B12^T was cultivated on MA plates and incubated in an anaerobic jar at 30°C for seven days, following the manufacturer's instructions (BD GasPak Systems). Susceptibility to antibiotics was tested on MA plates using various antibiotic discs (μg/disc): chloramphenicol (30), erythromycin (15), kanamycin (30), novobiocin (30), neomycin (30), penicillin (10 IU), tetracycline (30), and vancomycin (30) (Jorgensen and Turnidge, 2015).

2.4 Chemotaxonomic analysis

For the fatty acid analysis, cells of the strain WSW3-B12^T and the reference strains *Flexithrix dorotheae* KCTC 82993^T,

Rapidithrix thailandica KCTC 82795^T, and *Sediminitomix flava* KCTC 12970^T were collected from MA agar at the same physiological age. The standard MIDI protocol (version 6.2) was used to extract the fatty acids from the cell (Sasser, 1990). The extracted fatty acid methyl esters were then injected into a gas chromatograph and were analyzed by the Sherlock Microbial Identification System (TSBA; library version 6.0). For respiratory quinones, the compounds were extracted from bacterial biomass using the protocol of Komagata and Suzuki (Komagata and Suzuki, 1988). First, 100 mg of freeze-dried cells was treated with chloroform-methanol (2:1, v/v), and then crude compounds were separated by thin-layer chromatography (TLC, 20cm×20cm, silica gel 60 F254 plate, Merck) using petroleum benzene/diethyl ether (9:1, v/v) as the developing solvent. The quinone band was collected from a TLC plate and dissolved in acetone. Finally, the compound was purified and identified by reverse-phase high-performance liquid chromatography with a UV detector at 270 nm. Polar lipids were extracted from the freeze-dried cells by chloroform/methanol followed by two-dimensional TLC, as described by Minnikin et al. (Komagata and Suzuki, 1988). The two-dimensional TLC (10 cm×10 cm, silica gel 60 F254 plate, Merck) plates were run using chloroform-methanol-water (65:25:4, v/v/v) and chloroform-methanol-acetic acid-water (80:12:15:4, v/v/v/v) as the solvent systems for the first and second dimensions, respectively. Finally, the TLC plates were sprayed with 0.2% ninhydrin for the amino group-containing lipids, α-naphthol for sugar-containing lipids, molybdenum blue for phosphorus-containing lipids, 0.5% phosphomolybdic acid reagent for total lipids, and Dragendorff's solution for quaternary nitrogen-containing lipids.

2.5 Genome sequencing and analysis

The total genomic DNA of the strain WSW3-B12^T was extracted from two-day old culture on MA. The quality and quantity of the DNA samples were measured with a spectrophotometer (Nanodrop 2000/2000c), and the size and length were monitored by electrophoresis using 1% (w/v) agarose gel. The whole genome sequencing was performed by a combination of Illumina Novaseq 6000 and Oxford Nanopore Technologies (ONT) (at HME, Republic of Korea). For Illumina sequencing, the short-length DNA was used to build up a library while for the ONT high-molecular-weight DNA was used to prepare the library using a ligation sequencing kit (SQK-LSK109; ONT) following the manufacturer's protocol (version RPB_9059_v1_revC_08Mar2018). Sequencing was performed as multiplex runs on a MinION with MinKnow v1.15.1 using FLO-MIN106 R9.4 flow cells. Illumina reads were trimmed and filtered from raw reads using Trimmomatic (Bolger et al., 2014) and Filtlong v0.20 (https://github.com/rrwick/Filtlong) filtering tools. For Nanopore reads, the raw Fast5 files were demultiplexed with Guppy to retain the barcoded reads. Porechop v0.2.4 (https://github.com/rrwick/Porechop) was used to remove the barcode and adapters. The filtered Illumina and Nanopore reads were assembled using four different programs: Spades, CANU (Koren et al., 2017) Unicycler, and Flye (Lin et al., 2016). The quality initial genomes bins were then evaluated using CheckM (Parks et al., 2015).

2.6 Genome annotation and phylogeny

First, the genomes were annotated by NCBI Prokaryotic Genomes Automatic AnnotationPipeline (PGAAP) and Prokka version 1.14.6 (Seemann, 2014). The genome of the strain WSW3-B12^T contained 29 contigs. Each contig was predicted for plasmids and then blasted to find the most similar plasmids and their closest host using the PLSDB server v. 2021_06_23_v2 (Schmartz et al., 2022).

To determine the taxonomic position of the strain WSW3-B12^T the average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) were calculated using the ANI calculator tool in Kostas Lab Service (http://enve-omics.ce.gatech.edu/ani/) and the Genome to Genome Distance Calculator version 3.0 of DSMZ (https://ggdc.dsmz.de/ggdc.php#) (Meier-Kolthoff et al., 2013). For genus confirmation, amino acid identity (AAI) and percentage of conserved proteins (POCP) values were further calculated using Kostas Lab Service (http://enve-omics.ce.gatech.edu/aai/) and the BLASTP program (https://github.com/hoelzer/pocp), respectively (Qin et al., 2014). The whole-genome-based phylogenetic tree was built based on the alignment of 400 marker genes with FastTree using PhyloPhlAn version 3.0 (Asnicar et al., 2020). A nonmetric multidimensional scaling (nMDS) plot was made using a matrix of functional genes found in the COGs (Clusters of Orthologous Groups) database (Galperin et al., 2021), which was used to find the clustering of the strain WSW3-B12^T and the existing strains.

2.7 Genome functional analysis

The functional genes within each genome were annotated using the KEGG database and then translated into pathways using KEGG Decoder (Graham et al., 2018) and KEGG-Expander (https://github.com/bjtully/BioData/tree/master/KEGGDecoder). The prediction and classification of the orthologous groups associated with the protein coding genes were annotated using the COGs database (Galperin et al., 2021). The genomes were further annotated for functional genes using the rapid annotations based on subsystem technology (RAST) database (https://rast.nmpdr.org/rast.cgi). Carbohydrate active enzymes were annotated using the dbCAN2 meta server (Zhang et al., 2018). Biosynthesis gene clusters (BGCs) and metabolic gene clusters (MGCs) possessed by each genome were identified using antiSMASH 6.01 (Blin et al., 2019). The antimicrobial resistance (AMR) genes were predicted using the comprehensive antibiotic resistance database (CARD) database (Konaté et al., 2019; Alcock et al., 2022). The CRISPRCasFinder server (Couvin et al., 2018) and CRISPRcasIdentifier (https://github.com/BackofenLab/CRISPRcasIdentifier) were used to predict the CRISPR-Cas system (Padilha et al., 2020). Finally,

the cas enzyme's sequences were blasted using UniProt for confirmation (The UniProt Consortium, 2023). Phage sequences were identified by the PHAST (phage search tool) (Arndt et al., 2016).

3 Results and discussion

3.1 Isolation and identification

The strain WSW3-B12^T was isolated from the red alga genus *Chondrus* collected from tidal flats at the West Sea, Byeonsan, Republic of Korea. The strain grew well on MA and produced regular pin-pointed, light brown colonies after 48 hours' incubation. The diameters of the colonies ranged from 325-420 µm. Cells of the strain WSW3-B12^T were Gram-stain-negative, non motile and rod-shaped with a length of 5-7 µm and width of 0.46-0.54 µm (Figure S1).

3.2 16S rRNA gene analyses

The analysis of 16S rRNA gene sequences from the EzBioCloud server (http://www.ezbiocloud.net) revealed that the strain WSW3-B12^T was phylogenetically affiliated with the family *Flammeovirgaceae*, and the closest relatives were *Flexithrix dorotheae* DSM 6795^T (92.7%) and *Rapidithrix thailandica* TISTR 1750^T (90.8%). Furthermore, the phylogenetic trees based on 16S rRNA gene sequences showed clustering of the strain WSW3-B12^T with *Rapidithrix thailandica* TISTR 1750^T and *Flexithrix dorotheae* DSM 6795^T, (Figure 1). The closest strains *Flexithrix dorotheae* DSM 6795^T, *Rapidithrix thailandica* TISTR 1750^T, and *Sediminitomix flava* DSM 28229^T were isolated from marine silt, algae, and sediment, respectively (Hosoya and Yokota, 2007; Khan et al., 2007; Srisukchayakul et al., 2007). The strains *Flexithrix dorotheae* KCTC 82993^T, *Rapidithrix thailandica* KCTC 82795^T, and *Sediminitomix flava* KCTC 12970^T were used as reference strains. Finally, the 16S rRNA gene sequences of the strain WSW3-B12^T were submitted to the NCBI database under the accession number OP823709.

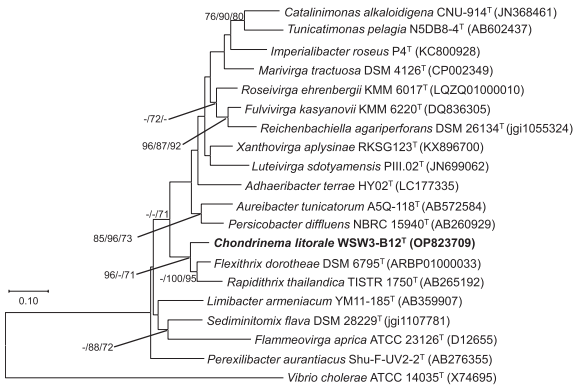

FIGURE 1
Maximum likelihood tree based on 16S rRNA gene sequences showing the phylogenetic relationship of the strain WSW3-B12^T and closely related genus of family *Flammeovirgaceae* and order *Cytophagales*. Bootstrap values (>70%) in the order of ML/NJ/MP are shown at the branch points based on 1000 replications. *Vibrio cholerae* ATCC 14035^T *were* used as outgroups. Bar 0.10 substitutions per nucleotide position.

3.3 Physiological and biochemical characteristics

The strain WSW3-B12^T was Gram-stain-negative, oxidase and catalase positive, strictly aerobic and long rod-shaped bacteria. Growth occurred in a temperature range of 15–30°C (optimum, 25–30°C), a pH range of 5.5–9.0 (optimum, 6.5–8.5), and a NaCl range of 1.0–7.0% (optimum, 2.0–5.0%) (w/v). The strain degraded cellulose, inulin, and starch but not chitin, κ-carrageenan, sodium alginate, and xylan. Cells were positive for the hydrolysis of Tweens 20, 40, and 80 but negative for the hydrolysis of casein and DNase. The strain was sensitive to chloramphenicol, novobiocin, tetracycline, and vancomycin, but resistant to erythromycin, kanamycin, neomycin, and penicillin. In the API ZYM test, activities of acid phosphatase, alkaline phosphatase, N-acetyl-β-glucosaminidase, cystine arylamidase, α-chymotrypsin, esterase (C4), esterase lipase (C8), α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase were present while activities of α-fucosidase, β-glucuronidase, lipase (C14), and α-mannosidase were absent. The strain was only positive for gelatinase in API 20E. In the API 50CH strip, acid is produced from L-arabinose, amidon (starch), arbutin, N- acetylglucosamine, D-cellobiose, gentibiose, D-galactose, glucose, D-lactose, methyl-αD-glucopyranoside, methyl-αD-mannopyranoside, D-maltose, D-melibiose, D-mannose, D-melezitose, D-raffinose, L-rhamnose, D-sucrose, D-trehalose, D-turanose, and D-xylose. In a GEN III MicroPlate (Biolog) test, cells utilize dextrin, L-fucose, D-glucose-6-phosphate, D-glucuronic acid, β-methyl-D-glucoside, L-malic acid, sucrose, D-trehalose, D-turanose, and quinic acid. Details of physiological and biochemical characteristics that differentiate the strain WSW3-B12^T from its closest relatives in the family *Flammeovirgaceae* are listed in (Table 1).

3.4 Chemotaxonomic analysis

The major fatty acids (>5.0%) of the strain WSW3-B12^T were iso-$C_{15:0}$ (24.9%), $C_{16:1}$ ω5c (23.8%), $C_{17:1}$ ω6c (9.4%), and iso-$C_{16:0}$ (7.9%). Meanwhile, iso-$C_{17:0}$ 3-OH (3.5%), summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*) (3.5%), $C_{15:0}$ 3-OH (3.2%), $C_{16.0}$ (2.8%), $C_{18:1}$ ω9c (2.3%), $C_{12:0}$ (2.0%), $C_{16:0}$ 3-OH (1.5%), and $C_{14:0}$ (1.4%) were the minor fatty acids (Table 2). The novel strain and all three reference strains showed a higher percentage of $C_{16:1}$ ω5c (15.3-41.0%) and iso-$C_{15:0}$ (8.5-24.9%) components of fatty acids. The strain WSW3-B12^T had a higher percentage of iso-$C_{16:0}$ (7.9%) and a lower percentage of $C_{16.0}$ (2.8%) as compared to the reference strains. Interestingly, among the four strains, only the strain WSW3-B12^T had $C_{17:1}$ ω6c (9.4%) component of fatty acid. The respiratory

10.3389/fmars.2023.1186809

TABLE 1 Differential characteristics of the strain WSW3-B12^T and phylogenetically closely related type species of the genus belongs to *Flammeovirgaceae*.

Characteristic	1	2	3	4
Temperature (°C)	15-30	17-40	25-30	10-37
pH optimum	5.5-9.0	6.5-8.0	5-10	6.0-9.0
NaCl (%, w/v)	1.0-7.0	0.5-5.0	0.5-7.0	0.5-5.0
Hydrolysis of Tween 40	+	+	–	+
Hydrolysis of Tween 80	+	+	–	+
Enzyme activity (API ZYM)				
N-Acetyl-β-glucosaminidase	+	–	–	–
α-Fucosidase	–	+	–	–
α-Galactosidase	+	+	–	–
β-Galactosidase	+	+	–	–
α-Glucosidase	+	–	–	–
β-Glucosidase	+	–	–	–
API 50CH				
Amygdalin	+	w	–	–
Amidon (starch)	+	w	–	+
Arbutin	+	–	–	–
N-Acetylglucosamine	+	–	+	–
D-Cellobiose	+	+	–	+
L-Fucose	–	–	+	–
D-Fructose	–	+	+	–
Glycogen	–	–	w	+
Glycerol	–	–	+	–
D-Galactose	+	+	+	–
Gentibiose	+	–	–	+
D-lactose	+	+	+	–
D-Mannose	+	+	+	–
Methyl-αD-glucopyranoside	+	+	–	–
Methyl-αD-mannopyranoside	+	+	–	–
D-Melezitose	+	–	–	–
D-Maltose	+	+	+	–
D-Melibiose	+	–	+	–
L-Rhamnose	+	+	–	–
D-Rafinose	+	–	–	–
D-Sucrose	+	+	–	–
D-Trehalose	+	+	+	–
D-Turanose	+	–	–	–
D-Xylose	+	+	+	–
GEN III MicroPlate assay (Biolog):				

(Continued)

TABLE 1 Continued

Characteristic	1	2	3	4
Acetic acid	–	+	+	–
L-Aspartic acid	–	+	+	–
Aztreonam	–	+	+	–
N-Acetyl-β-D-mannosamine	–	+	+	+
N-Acetyl-neuraminic acid	–	+	+	–
D-Cellobiose	–	–	–	+
Dextrin	+	+	+	–
D-Glucuronic acid	+	+	+	–
D-Glucose-6-PO4	+	–	–	–
Glycerol	–	–	+	–
Gelatin	–	–	+	–
L-Glutamic acid	–	+	+	–
α-D-Lactose	+	–	–	–
β-Methyl-D-glucoside	+	+	+	–
D-Mannose	–	–	+	+
L-Malic acid	+	–	–	–
Nalidixic acid	+	–	–	–
Quinic acid	+	–	–	–
Sucrose	+	–	+	–
1% Sodium Lactate	–	–	+	–
D-Serine	–	–	+	–
L-Serine	–	+	+	–
Tetrazolium violet	–	–	+	–
Tetrazolium blue	–	–	+	–

Strain: 1, WSW3-B12^T; 2, *Flexithrix dorotheae* KCTC 82993^T; 3, *Rapidithrix thailandica* KCTC 82795^T; 4, *Sediminitomix flava* KCTC 12970^T All strains were positive for the hydrolysis of starch and Tween 20 and for the activities of oxidase and catalase. All the data are from this study except for the temperature and pH determination of the reference strains are from the literature (Hosoya and Yokota, 2007; Khan et al., 2007; Srisukchayakul et al., 2007). +, Positive; –, negative; w, weak.

quinones of the strain WSW3-B12^T was menaquinone 7 (MK-7) which was also founded in the reference strains. The polar lipids of the strain WSW3-B12^T contained phosphatidylethanolamine, one unidentified aminophospholipid, four unidentified glycolipids, and three unidentified lipids (Figure S2).

3.5 Genomic general features

The combined Nanopore and Illumina sequencing platforms were used to determine the complete genomes of the strains WSW3-B12^T. The CheckM analysis showed that the strain had 99.4% completeness and only 1.45% contamination, which indicated the high quality and reliability of the genomes. The complete genome of the strain WSW3-B12^T comprises 29 contigs, among which contig 1 was a circular chromosome length of 4.938441 bp while the remaining 28 contigs were plasmids, out of which six were mobile plasmids and the rest were non-mobilizable plasmids. Some genomes of the family *Flammeovirgaceae*, the phylum *Bacteroidota* are relatively large (Feng et al., 2021a): the genomes of *Flammeovirga yaeyamensis* NBRC 100898 (Feng et al., 2021b), *Flammeovirga* sp. SJP92 (Dong et al., 2017), *Flexithrix dorotheae* DSM 6795^T, and *Flammeovirga aprica* JL-4^T are 7.4, 8.4, 9.5, and 9.3 Mbp, respectively. The details of each plasmid are summarized in Table S1. Interestingly, limited numbers of bacteria have been discovered to have multiple plasmids (Cazares et al., 2020, Feng et al., 2021b). Some marine members of the phylum *Bacteroidota* carry a single large plasmid (Zhong et al., 2001; Saw et al., 2012). We hypothesize that the strain WSW3-B12^T received plasmids from other bacteria in the environment, which explains the presence of the high number of plasmids and the mobile genetic elements in the genome of the strain WSW3-B12^T.

Genome annotation by PGAAP pipeline in the NCBI identified a total of 8199 genes, nine ribosomal RNA genes (5S, 3; 16S, 3; 23S, 3), and a total of 51 transfer RNA genes. The G+C content of the strain WSW3-B12^T was 34.1%, while for the reference strains *Flexithrix*

TABLE 2 Cellular fatty acid compositions (%) of the strain WSW3-B12^T and phylogenetically closely related type species of genus belonging to *Flammeovirgaceae*.

Fatty acid (%)	1	2	3	4
Saturated				
$C_{10:0}$	–	–	1.2	–
$C_{12:0}$	2.0	–	–	–
$C_{14:0}$	1.4	1.2	2.2	5.1
$C_{16:0}$	2.8	8.5	12.5	8.0
$C_{18:0}$	–	1.5	3.9	–
Unsaturated:				
$C_{16:1}\omega5c$	23.8	41.0	36.2	15.3
$C_{18:1}\omega9c$	2.3	–	6.4	–
$C_{17:1}\omega6c$	9.4	–	–	–
Branched:				
iso-$C_{11:\ 0}$	–	–	1.7	–
iso-$C_{15:0}$	24.9	12.8	8.5	22.4
iso-$C_{15:1}$ G	–	–	–	2.3
anteiso-$C_{15:\ 0}$	–	1.4	–	1.3
iso-$C_{16:0}$	7.9	–	2.3	1.7
iso-$C_{17:0}$	–	–	2.5	–
Hydroxy:				
iso-$C_{15:0}$ 3-OH	–	4.33	3.0	1.9
$C_{15:0}$ 3-OH	3.2	–	–	1.3
$C_{16:0}$ 3-OH	1.5	3.6	2.7	17.2
iso-$C_{16:0}$ 3-OH	–	–	–	1.1
iso-$C_{17:0}$ 3-OH	3.5	10.2	8.4	2.3
Summed features:*				
2	–	–	–	1.7
3	3.5	10.3	2.1	13.0
4	2.7	2.6	5.7	–
9	–	–	–	2.7

Summed features 2, ($C_{14:0}$ 3-OH/$C_{16:0}$ iso I); Summed features 3, ($C_{16:0}$ ω7c/$C_{16:10}$ ω6c); Summed features 4, ($C_{17:\ 0}$ iso I/anteiso B); summed feature 9, ($C_{16:0}$ 10-methyl and/or iso-$C_{17:0}$ x9c) Strain: 1, WSW3-B12^T; 2, *Flexithrix dorotheae* KCTC 82993^T; 3, *Rapidithrix thailandica* KCTC 82795^T; 4, *Sediminitomix flava* KCTC 12970^T All data are from this study. Numbers indicate the percentages of the fatty acids. -, not detected (<1%).

dorotheae DSM 6795^T and *Sediminitomix flava* DSM 28229^T the content was 36.4 and 35.6%, respectively. The genomic features of the strain WSW3-B12^T and the existing strains are presented in (Table 3). The genome of the strain WSW3-B12^T was submitted to the NCBI with the GenBank accession number GCA_026250525.

3.6 Whole-genome-based phylogeny

To further confirm the taxonomic position of the strain WSW3-B12^T, the ANI and dDDH were calculated among the novel strain and type species of four genus of *Flammeovirgaceae Flexithrix dorotheae* DSM 6795^T, *Sediminitomix flava* DSM 28229^T, *Flammeovirga aprica* JL-4^T, and *Xanthovirga aplysinae* RKSG123^T. The ANI and dDDH between the strain WSW3-B12^T and *Flexithrix dorotheae* DSM 6795^T, *Sediminitomix flava* DSM 28229^T, *Flammeovirga aprica* JL-4^T, and *Xanthovirga aplysinae* RKSG123^T were ranged from 72.3-76.6 and 19.2-26.1%, respectively. The values of ANI and dDDH were below the standard cut-off values for speciation, which are ANI (95-96%) (Yoon et al., 2017b) and dDDH (70%) (Auch et al., 2010). The AAI and POCP values between the strain WSW3-B12^T and *Flexithrix*

TABLE 3 Genome features of the strain WSW3-B12^T and its closest related members in the family *Flammeovirgaceae.*

	1	2	3	4
Accession no.	GCA_026250525	NZ_ARBP00000000	NZ_QGDO01000001	NZ_JABANE010000001
Approximate genome size (Mbp)	10.1	9.5	6.6	9.3
G+C content (%)	34.1	36.4	35.6	35.2
Completeness	99.4	99.4	99.0	98.8
Contamination	1.45	0.6	2.98	3.21
Total number of genes	8199	7205	5059	7020
rRNAs (5S, 16S, 23S)	3,3,3	2,2,2	7,3,2	1,4,6
tRNAs	51	44	107	75
Pseudo Genes	69	51	11	153

Strain: 1, WSW3-B12^T; 2, *Flexithrix dorotheae* DSM 6795 ^{T}; 3, *Sediminitomix flava* DSM 28229 ^{T}; 4, *Flammeovirga aprica* JL-4 ^{T}.

dorotheae DSM 6795^T, *Sediminitomix flava* DSM 28229^T, *Flammeovirga aprica* JL-4^T, and *Xanthovirga aplysinae* RKSG123 ^{T} were ranged from 46.79-57.2 and 28.3-55.6%, respectively. The AAI cut-off value applied for the delineation of genus has a wide range of 60–80% (Park et al., 2022). A POCP value of 50% can be used for genus delineation, with a 55% value as the genus boundary (Qin et al., 2014; Barco et al., 2020). Thus, the strain WSW3-B12^T could represent a novel genus because genome-relatedness values are significantly lower than the species and genus boundaries. The phylogenomic tree showed that the strain WSW3-B12^T formed a clade with the closest strains, *Flexithrix dorotheae* DSM 6795^T and *Sediminitomix flava* DSM 28229^T, which also belongs to *Flammeovirgaceae* (Figure 2). The nonmetric multidimensional scaling (nMDS) plots further presented the same cluster between the strains WSW3-B12^T and *Flexithrix dorotheae* DSM 6795^T (Figure S3).

3.7 Genome functional analysis

3.7.1 Metabolic pathways analysis

First, a comparison of the distribution of genes in the KEGG module pathways for the strain WSW3-B12^T and existing stains is presented in a heatmap (Figure 3). The figure shows that the central metabolic pathways that are typically involved in aerobic respiration, sugar metabolism, amino acid synthesis, etc. appeared to be similar among these strains. The strain WSW3-B12^T contains genes that are involved in the biosynthesis of vitamins such as cobalamin, thiamin, and retinal. Furthermore, the KEGG pathways showed that the strain harbors genes for various enzymes, among which the genes for oligogalacturonide lyase (OGL) were only found in the genome of WSW3-B12^T. Interestingly, *OGL* genes exists on plasmid 3 instead of the main chromosome. This makes strain WSW3-B12^T unique compared to other *Flammeovirgaceae* genera that do not have the OGL genes. The *OGL* genes may give the strain WSW3-B12^T evolutionary advantages compared to other microorganisms. The *OGL* genes catalyzed the eliminative removal of unsaturated terminal residues from oligosaccharides of D-galacturonate, which is a key constituent of pectin in plant biomass (Linhardt et al., 1987; Abbott and Boraston, 2008; Abbott et al., 2010). The *OGL* genes is present in a group of pectinolytic bacteria, such as *Erwinia* species, that are involved in the

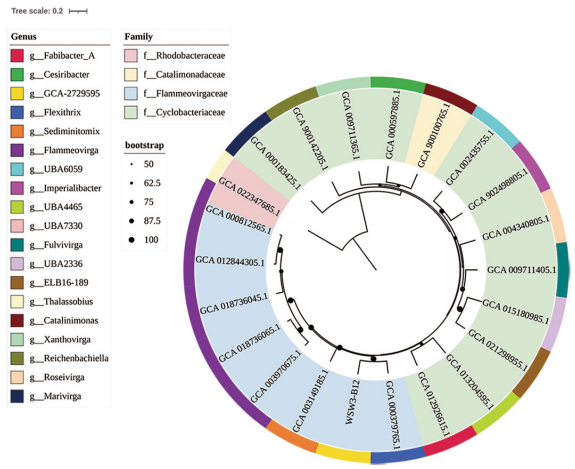

FIGURE 2
A whole-genome-based phylogenetic tree based on 400 marker genes using PhyloPhlAn version 3.0 shows the position of the strain WSW3-B12^T among the members of the families *Flammeovirgaceae, Catalimonadaceae*, and *Cyclobacteriaceae* in the order *Cytophagales.* The strain *Thalassobius* sp. Cn5-15 (GCA_022347685) of family *Rhodobacteraceae* was used as an outgroup. Strains: WSW3-B12^T, *Flexithrix dorotheae* DSM 6795^T (GCA_000379765), *Marinigracilibium pacificum* KN852 (GCA_012926615), *Flammeovirgaceae* sp. bin.302 (GCA_013204595), *Flammeovirgaceae* sp. CoA8N_M18 (GCA_021298955), *Flammeovirgaceae* sp. H1_BAC1 (GCA_015180985), *Fulvivirga kasyanovii* JCM 16186 ^{T} (GCA_009711405), *Roseivirga ehrenbergii* DSM 102268 ^{T} (GCA_004340805), *Imperialibacter* sp. EC-SDR9 ^{T} (GCA_902498805), *Flammeovirgaceae* sp. UBA6059 (GCA_002435755), *Catalinimonas alkaloidigena* DSM2516^T (GCA_900100765), *Flammeovirgaceae* sp. 311 (GCA_000597885), *Xanthovirga aplysinae* RKSG123^T (GCA_009711365), *Reichenbachiella agariperforans* DSM 26134^T (GCA_900142205), *Marivirga tractuosa* DSM 4126^T (GCA_000183425), *Thalassobius* sp. Cn5-15 (GCA_022347685), *Flammeovirga* sp. OC4 (GCA_000812565), *Flammeovirga aprica* JL-4^T (GCA_012844305), *Flammeovirga yaeyamensis* (GCA_018736045), *Flammeovirga kamogawensis* (GCA_018736065), *F pectinis* (GCA_003970675), *Sediminitomix flava* DSM 28229^T GCA_003970675.

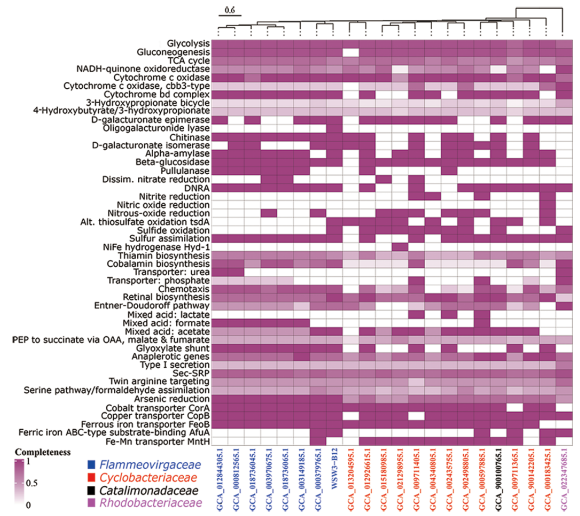

FIGURE 3
Heatmap of discriminated metabolic pathways within the genome of the strain WSW3-B12^T and 21 representative genomes of the existing members of the *Flammeovirgaceae, Catalimonadaceae*, and *Cyclobacteriaceae* in the order *Cytophagales* as used in Figure 2. The cell indicates the completeness of each pathway referring to annotations using KEGG. The heatmap of metabolic pathways was created using the same strains as in Figure 2 for a comparative study.

degradation of pectin, resulting in characteristic plant cell necrosis and tissue maceration (Collmer and Bateman, 1981).

Second, the COG analysis showed that the major genes were those involved in general function prediction, followed by signal transduction mechanisms, while the minor genes were those involved in cytoskeleton formation (Figure 4). The COG analysis further showed that genes for signal transduction were higher in the strain WSW3-B12^T compared to existing strains. Microbes use

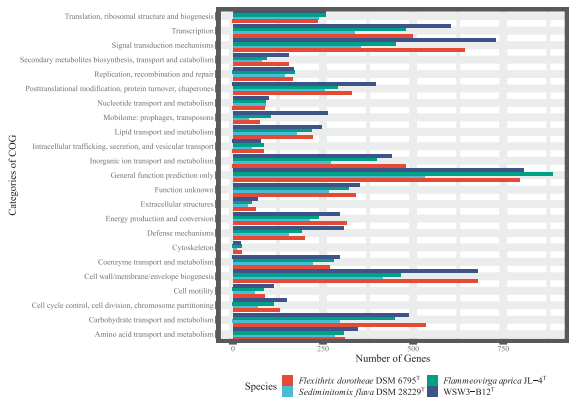

FIGURE 4
Clusters of Orthologous Group (COG), functional classification of proteins in the genome of the WSW3-B12^T and three existing strains of *Flammeovirgaceae*.

multiple signal transduction systems to respond to environmental changes and mediate diverse physiological processes and intercellular communication, including antibiotic resistance, virulence, symbiotic bacteria-host interactions, and biofilm assemblage (Wang et al., 2021). In the RAST system, a total of 8525 protein-encoding genes were predicted and assigned to 275 subsystems. In the subsystem category, the highest number of genes were assigned to the metabolism of amino acids and derivatives (303) and carbohydrates (251), followed by cofactors, vitamins, prosthetic groups, and pigment metabolism (171). Although the distribution of genes between novel strains and the closest strains was similar. The numbers of genes involved in regulation/cell signaling and cell wall/capsule synthesis were higher in the strain WSW3-B12^T than in the other strains (Table S2). Bacteria use these regulatory and cell signaling pathways for intercellular communication and diverse biological processes including biofilm formation (Wang et al., 2021). Furthermore, the higher number of cell wall and capsule synthesis pathways further helps the strain WSW3-B12^T in unfavorable conditions as well as in interaction of the strain with algae where the strain is isolated from.

The genes for polysaccharide degradation were identified using dbCAN meta servers, which detected a number of CAZyme gene clusters (CGCs) in the genome of the strain WSW3-B12^T and the existing strains. The strain WSW3-B12^T carried a total of 241 CAZymes. The distribution of CAZyme gene clusters (CGCs) between the novel and existing strains is summarized in (Table S3). The percentage of CAZyme genes out of the total genes and the ratio of the number of glycoside hydrolases (GHs) per Mbp genome were calculated. The strain WSW3-B12^T carried 2.94% CAZym and 13.86 GHs per Mbp of genome (Table S4). GHs and PLs that are expected to participate in degradation of polysaccharide were searched from the dbCAN meta server and compared in terms of the *in vitro* activities of the strain WSW3-B12^T (Table S5). The strain degraded cellulose, inulin, and starch. The strain carried four GH3 and five GH5 for cellulose degradation, one GH32 for inulin degradation, and eight GH13 for starch degradation. These GHs may participate in the degradation of the test polysaccharides. The strain did not carry any genes for the degradation of agar and alginate while it carried two genes for the degradation of κ-carrageenan.

3.7.2 Metabolism of heavy metals

The KEGG pathways showed that the strain WSW3-B12^T and the existing strain of *Flammeovirgaceae* had complete pathways for arsenic reduction (Figure 3) and also carried transporter genes such as *Cor*A, *CopA*, *FeoB*, and *AfuA* for cobalt, copper, ferrous, and ferric iron, respectively. The *CorA* proteins are generally associated with the transport of magnesium ions, but some members of the *CorA* family can also transport other ions such as cobalt (Co) and nickel (Ni) (Zhang et al., 2009). The strain WSW3-B12^T carried *CorA* in both chromosome and plasmid 11. *CopA* is one of the most well-known microbial metal transporters. The monomer of *CopA* binds to two Cu^{2+} ions and subsequently transfers them to the periplasm using the Cu chaperone (CusF) and ATP energy (Li et al., 2022). For the transportation of ferrous and ferric iron, the strain carries the FeoB protein, a cytoplasmic membrane transporter

protein, and *AfuA*, an ABC-type transporter protein. The ABC transporters help the microbes in membrane translocation of HMs ions (Hantke, 2003; Lau et al., 2016). Interestingly, *FeoB* genes exist on both chromosome and plasmid 6. Plasmid 6 even has two *FeoB* genes, compared to only one on the chromosome (Figure S5).

More HMs metabolism genes were discovered on the chromosome and in the plasmids as a result of the Prokka annotation. Many iron and copper metabolic genes were distributed in both the chromosome and the plasmids proportionally. Meanwhile, most of the arsenic and cobalt metabolic genes come from the plasmids. Thus, the plasmid has an interesting relationship with heavy metal metabolism. Details of these genes are listed in (Table S6).

3.7.3 Prediction of biosynthetic gene clusters and antibiotic resistance genes

The strain possessed six types of putative BGCs (terpene, type I polyketide synthases, type III polyketide synthases, betalactone, acyl amino acid, and lanthipeptide class I). Compared to the novel strain, only two BGCs in *Flexithrix dorotheae* DSM 6795^T, three BGCs in *Sediminitomix flava* DSM 28229^T, and five BGCs in *Flammeovirga aprica* JL-4^T were detected. (Table S7). Among the BGCs, the polyketide synthases are a family of multi-domain enzymes that produce polyketides, a large class of antimicrobial secondary metabolites (Hochmuth and Piel, 2009), while lanthipeptide class I are ribosomally synthesized antimicrobial compounds that are also called lantibiotics. Lanthipeptide can be used as an alternative to antibiotics in the food industry and veterinary medicine, and against multi-drug resistant strains methicillin-resistant Staphylococcus aureus.(MRSA) and vancomycin-resistant enterococcus (VRE) (Barbosa et al., 2015).

Based on CARD database prediction, there are a total of four antibiotic resistance genes, one on the chromosome and three on the plasmids (plasmid 3, 5, and 9). The chromosome contains a glycopeptide antibiotic resistance gene cluster and the plasmids contain fluoroquinolone and tetracycline antibiotic resistance.

3.7.4 CRISPR-Cas system and prophage analysis

CRISPR-Cas system consists of CRISPR array (spacers and repeats) and Cas system (collection of Cas enzymes). First, the CRISPRCasFinder was used to detect the CRISPR array. CRISPRcasIdentifier was then used to identify CRISPR related enzymes in the genome. The sequences were then blasted using UniProt for confirmation (Table S8). The CRISPRCasFinder server detected seven CRISPR arrays candidates in the genome of the strain WSW3-B12^T. However, only one CRISPR array, located in plasmid 20, that are long enough to be consider CRISPR array. The CRISPRcasIndetifier and Uniprot also only detects Cas enzymes in the plasmid 20 upstream of the CRISPR array. There were eight Cas enzymes, Cas1 to 7 and an unknown Cas enzyme (Figure S4). Thus, the CRISPR/Cas system could be classified as an incomplete Class I type I-B that is missing the Cas8 enzyme (Makarova et al., 2020). Based on this analysis we confirmed that the strain WSW3-B12^T have the CRISPR-Cas system in the plasmid 20.

When we compared the genome of the strain WSW3-B12^T with that of other reference strains, only the genome of *Sediminitomix flava* DSM 28229^T contained two CRISPR arrays. The other reference strains did not have a CRISPR array. When we searched similar sequences from Uniprot, the highest sequence identity of Cas3, 5, 6, and 7 of the strain WSW3-B12^T were found from the Cas genes of *Sediminitomix flava* DSM 28229^T (Table S8). *Sediminitomix flava* DSM 28229^T contains only Cas 3, 5, 6, and 7 on its chromosome. Thus, it also has an incomplete Class I type I-B CRISPR/Cas system. It is very likely that the Cas enzymes (3, 5, 6, and 7) of strains WSW3-B12^T and *Sediminitomix flava* DSM 28229^T came from the same lineage. Based on their isolation sites, this is also probable because strain WSW3-B12^T was isolated from the West Sea, Korea and *Sediminitomix flava* DSM 28229^T was isolated from Okinawa, Japan: both locations are connected by a sea current.

The PHAST search tool identified two prophage regions, one of which is incomplete and the other is questionable. The high number of CRISPRs candidates per strain described as the prokaryotic defense mechanism against external attacks and the presence of bacteriophages in their genomes suggest that the strain is also prone to phage infections. The CRISPR-based technologies are also useful for either sequence-specific killing of pathogenic bacteria or for the removal of accessory genes (antimicrobial resistance, virulence, etc.) (Palacios Araya et al., 2021).

3.7.5 Plasmid functional analysis

The function of the chromosome and each plasmid were analyzed using the KEGG database. Based on this analysis we classified the pathways into three categories based on their locations: found exclusively in the chromosome (Figure S5A); found both in the chromosome and plasmids (Figure S5B); and found exclusively in the plasmids (Figure S5C). The existence of plasmids in addition to the chromosome could help the strain WSW3-B12^T to have more robust and highly expressed metabolic pathways. The addition of plasmids may increase the competitive edge of the strain WSW3-B12^T in terms of nutrition, growth, and defense in the marine environments. For example, the chromosome of the strain WSW3-B12^T lacked cysteine pathways but plasmids 3 and 11 carried genes for the cysteine pathway that may supplement cysteine metabolism (Figure S5C).

3.8 Description of *Chondrinema* gen. nov.

Chondrinema (Chon.dri.ne'ma. N.L. masc. n. Chondrus, a genus of red algae; Gr. neut. n. nema, a thread; N.L. neut. n. Chondrinema, a thread associated with the algal genus Chondrus).

This genus falls within the phylum *Bacteroidota*, class *Cytophagia*, and order *Cytophagales*. Cells are Gram-stain negative, oxidase and catalase-positive, and aerobic. The major fatty acids of the strain are iso-$C_{15:0}$ and $C_{16:1}$ ω5. The major respiratory quinone and polar lipid are menaquinone 7 (MK-7) and phosphatidylethanolamine.

3.9 Description of *Chondrinema* litorale sp. nov.

Chondrinema litorale (li. to. ra'le. L. neut. adj. *litorale*, of or belonging to the seashore)

Cells grow well on MA and produce light brown colonies with a diameter in a range from 325-420 μm. Cells of the strain are rod-shaped with a length 5-7 μm and width 0.46-0.54 μm.

Growth occurs at 15-30°C (optimum, 20-30°C), at pH 5.5-9.0 (optimum, pH 6.5-8.0), and with 1-7.0% NaCl (w/v) (optimum, 2-5%). The cell can degrade cellulose, inulin, and starch. Cells are positive for the hydrolysis of Tweens 20, 40, and 80. They are also positive for the activities of acid phosphatase, alkaline phosphatase, N-acetyl-β-glucosaminidase, cystine arylamidase, α-chymotrypsin, esterase (C4), esterase lipase (C8), α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, leucine arylamidase, naphthol-AS-BI-phosphohydrolase, trypsin, and valine arylamidase. The cell can produce acid from L-arabinose, amidon (starch), arbutin, N-acetylglucosamine, D-cellobiose, gentibiose, D-galactose, glucose, D-lactose, methyl-αD-glucopyranoside, methyl-αD-mannopyranoside, D-maltose, D-melibiose, D-mannose, D-melezitose, D-raffinose, L-rhamnose, D-sucrose, D-trehalose, D-turanose, and D-xylose.

The type strain WSW3-B12^T (= KCTC 82707^T = GDMCC 1.3198^T) was isolated from a red algae genus (*Chondrus*). The size of the genome is 10.1 Mbp and the G+C content is 34.1%. The genome contains one circular chromosome and 28 plasmids.

Repositories: The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WSW3-B12^T is OP823709 and that for the whole genome sequence is GCA_026250525.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: GenBank, OP823709.1 and GCA_026250525.1.

Author contributions

NM and S-GK designed the study. NM performed the isolation and the morphological, physiological, and biochemical characterization. FA and Y-JL performed bioinformatic analyses with help from K-HK and HLH. NM, FA and S-GK wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1186809/full#supplementary-material

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