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Comparative genomic analysis of the genus *Marinomonas* and taxonomic study of *Marinomonas algarum* sp. nov., isolated from red algae *Gelidium amansii*

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Abstract

Members of the genus *Marinomonas* are known for their environmental adaptation and metabolically versatility, with abundant proteins associated with antifreeze, osmotic pressure resistance, carbohydrate and multiple secondary metabolites. Comparative genomic analysis focusing on secondary metabolites and orthologue proteins was conducted with 30 reference genome sequences in the genus *Marinomonas*. In this study, a Gram-stain-negative, rod-shaped, non-flagellated and strictly aerobic bacterium, designated as strain E8T, was isolated from the red algae (*Gelidium amansii*) in the coastal of Weihai, China. Optimal growth of the strain E8T was observed at temperatures 25–30 °C, pH 6.5–8.0 and 1–3% (w/v) NaCl. The DNA G + C content was 42.8 mol%. The predominant isoprenoid quinone was Q-8 and the major fatty acids were C16:0, summed feature 3 and summed feature 8. The major polar lipids were phosphatidylglycerol (PG) and phosphatidylethanolamine (PE). Based on data obtained from this polyphasic taxonomic study, strain E8T should be considered as a novel species of the genus *Marinomonas*, for which the name *Marinomonas algarum* is proposed. The type strain is E8T (= KCTC 92201T = MCCC 1K07070T).

Keywords Algae epiphytic bacteria · Comparative genomic analysis · *Marinomonas* · Oceanospirillaceae · Polyphasic taxonomy · Secondary metabolites

Abbreviations

AAI Amino Acid Identity
ANI Average Nucleotide Identity
BD Becton Dickinson
HPLC High Performance Liquid Chromatography
KCTC Korean Collection for Type Cultures
MCCC Marine Culture Collection of China

MEGA Molecular Evolutionary Genetics Analysis
MIDI Microbial Identification System
TLC Thin Layer Chromatography

Introduction

The genus *Marinomonas* belongs to the family *Oceanospirillaceae* in the *Gammaproteobacteria*, was created by Landschoot and Ley (1984) to accommodate two reclassified species, namely *Marinomonas vaga* and *Marinomonas communis*. At the time of April of 2022, 39 validly published species (https://lpsn.dsmz.de/genus/marinomonas) had been isolated from the marine environment, including 11 species from marine plants, 10 from seawater and 5 from marine sediment. Many of them had been observed special properties like agar degradation (*M.agarivorans* KCTC 52475T) (Yu et al. 2020), algicidal activity (*M.algicida* KEMB 9005-327T) (Kristyanto et al. 2017), plant-beneficial properties (*M.spartinae* KCTC 42958T) (Lucena et al. 2016) and thermophilic characteristic (*M.primoryensis* JCM 92201T).
Members of the genus *Marinomonas* are Gram-stain-negative, rod-shaped, strictly aerobic, some of them could hydrolyze agar and gelatin, and have the ability to metabolize quinate, acetate, glycerol, and lactate (Ivanova et al. 2005; Lucas-Elio et al. 2011; Yu et al. 2020). Previous studies have verified that members of the genus *Marinomonas* show genomic, proteomic, and physiological adaptations to challenging marine environments (Vance et al. 2014; Liao et al. 2021). Varied kinds of secondary metabolites have been tracked in some strains in the genus *Marinomonas*, including α-ester-containing peptides (OEPs) (as a kind of RiPPs) from *Marinomonas fungiae* JCM 18476<sup>T</sup> (Kaweewan et al. 2021), marinocine from *Marinomonas mediterranea* MMB-1 (Lucas-Elio et al. 2005) and antimicrobial potentials (Wang et al. 2016).

In this study, a novel species within the genus *Marinomonas* was isolated from the surface of marine red algae and polyphasic taxonomy method was used to identify the taxonomic position of the novel strain E8<sup>T</sup>. We have preliminarily analysed the pan-genomes, orthologue proteins in the genus and predicted the biosynthetic gene clusters related to secondary metabolism.

**Materials and methods**

**Isolation, cultivation and maintenance**

The strain E8<sup>T</sup> was isolated from marine red algae (*Gelidium amansii*) collected from the coast of XiaoShi Island Weihai, China (37.5°N, 122.1°E) during a low tide and brought to the laboratory in a cold sterilized chamber. Samples were smashed into small pieces and homogenized. Then 1 g homogenate was weighed out and blended in 9 ml sterilized seawater and mixed homogeneously as 10<sup>–1</sup>. After gradient dilution to 10<sup>–2</sup> and 10<sup>–3</sup>, 100 µl of three dilutions were spread on marine agar 2216 (MA, Becton Dickinson) and incubated at 25 °C for 5 days. A single colony of strain E8<sup>T</sup> was obtained in pure culture after transferring to fresh MA media supplemented with 1% (v/v) NaCl. The phylogenetically related reference strains *Marinomonas communis* JCM 20766<sup>T</sup> and *Marinomonas pontica* DSM 17793<sup>T</sup>, were obtained from Pro. Du. All sampling and isolating experiments were performed in the April of 2021.

**16S rRNA gene sequence and phylogenetic analysis**

To identify the taxonomic position of strain E8<sup>T</sup>, the 16S rRNA gene sequence was amplified by PCR using two universal primers 27f and 1492r (Liu et al. 2014). The purified PCR products were ligated to the pGM-T vector and transferred into E. coli DH5α cells. The positive clones were selected and performed sequencing by BGI Co. Ltd. (Qingdao, PR China) using the ABI 3730XL system. The obtained 16S rRNA gene sequence of strain E8<sup>T</sup> was submitted to the GenBank database and the 16S rRNA gene sequence similarities were calculated using the BLAST algorithm at NCBI (https://www.ncbi.nlm.nih.gov) and EzBioCloud (http://www.ezbiocloud.net/) (Yoon et al. 2017). The MEGA version 7.0 (Kumar et al. 2016) was used to reconstruct phylogenetic trees with the neighbor-joining, maximum-likelihood and maximum-parsimony algorithms. The stability of the topology was confirmed by performing bootstrap analyses based on 1000 replications (Felsenstein 1985).

**Genome sequencing and function analysis**

The genomic DNA of strain E8<sup>T</sup> was extracted from a culture grown in MB (Becton Dickinson) for 48 h using a Bacteria DNA Kit (Omega) according to the manufacturer’s recommendations and then the purified DNA was sent to Beijing Novogene Bioinformatics Technology Co., Ltd. The sequencing library was prepared using NEBNext™ Ultra<sup>™</sup> DNA Library Prep Kit for Illumina (NEB, USA) following the manufacturer’s recommendations and sequenced using the pair-end 350 bp sequencing protocol on the Illumina PE150 platform. To obtain clean data, raw sequencing data were generated using refdqi (Version 10) at first and Illumina base-calling software CASAVA v1.8.2 (http://www.support.illumina.com/) according to its corresponding manuscript. All good quality paired reads were assembled using SOAPdenovo software (Li et al. 2008). To make sure the authenticity of 16S rRNA, the complete 16S rRNA gene sequence which was accessed from the draft genome using the RNAmer 1.2 server (http://www.cbs.dtu.dk/services/RNAmer/) had been compared with PCR amplification. The G+C content of the chromosomal DNA was calculated using genome sequence. The genome component prediction was conducted with GeneMarkS program (Besemer et al. 2001), tRNAscan-SE software (Version 1.3.1) (Lowe and Eddy 1997), rRNAmer software (Version 1.2) (Lagesen et al. 2007), Rfam database (Gardner et al. 2009), Island-Path-DIOMB program (Hsiao et al. 2003), PHAST software (Version 2.3) (Zhou et al. 2011) and CRISPRFinder (Grissa et al. 2007) to predict the related coding genes, tRNA, rRNA, snRNA, Genomics Islands, prophage and Clustered Regularly Interspaced Short Palindromic Repeat Sequences (CRISPR), respectively. Then, a whole genome Blast search (E value less than 1e-5, minimal alignment length percentage larger than 40%) was performed to predict gene function by using Gene Ontology (GO) (Ashburner et al. 2000), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2015), Clusters of Orthologous Groups (COG) (Galperin et al. 2015), Rapid Annotations using Subsystem (RAST) (Aziz et al. 2008) and Biological Process (GO) (Ashburner et al. 2000) databases.
Technology (Rastekenari et al.) server (Aziz et al. 2008) and Transporter Classification Database (TCDB) (Saier et al. 2014). To further detect the taxonomic relationship between the strain E8T and within members in the genus Marinomonas, phylogenetic analyses based on genomes inferred bac120 marker set via GTDB-Tk (Chaumeil et al. 2020) were reconstructed by using FastTree (Price et al. 2010) with JTT+CAT parameters and performed using IQ Tree (Trifinopoulos et al. 2016) with the LG+F+I+G4 model and 1000 bootstrap replicates.

**Morphological, physiological, and biochemical analysis**

The morphological and physiological features of strain E8T were examined with cells grown on MA at 28 °C for 48 h. Cell morphology, size and the presence of flagella were examined by light microscopy (E600, Nikon) and transmission electron microscopy (JEM-1200; JEOL) at the State Key Laboratory of Bio-Fibers and Eco-Textiles (Qingdao University, China). Gram staining was performed as the method described previously (Smibert and Krieg 1994). Gliding motility was tested in marine broth 2216 (MB; BD) supplemented with 0.3% agar and motility was examined using the hanging-drop method according to the methods of Bernardet et al. (2002). Cells of strain E8T were incubated on MA for 14 days with or without 0.1% (w/v) KNO₃ in an anaerobic jar to determine the growth under anaerobic conditions (10% H₂, 10% CO₂ and 80% N₂). Growth ranges and optimum temperature were indicated on MA at 4, 10, 15, 20, 25, 28, 30, 33, 37, 40 and 45 °C. NaCl range for growth were tested in the following medium (per liter: 1 g yeast extract, 5 g peptone, 0.1 g ferric citrate), supplying with artificial seawater (per liter: 3.2 g MgSO₄, 2.2 g MgCl₂, 1.2 g CaCl₂, 0.7 g KCl, 0.2 g NaHCO₃) containing 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10% (w/v) NaCl at 28 °C, and recorded the colony growth every 12 h. The pH range for growth was tested in marine broth 2216 (MB; BD) with addition of appropriate buffers, including MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.5), HEPES (pH 7.5 and 8.0), Tricine buffer (pH 8.5) and CAPSO (pH 9.0, 9.5 and 10.0), at a concentration of 20 mM and monitored the growth of bacterial using a spectrophotometer at 600 nm. The oxidase activity and catalase activity were assessed by using an oxidase reagent kit (bioMérieux) according to the manufacturer’s instructions and the production of bubbles after the addition of a drop of 3% (v/v) H₂O₂. The nitrate reduction and degradation of agar, starch, cellulose, alginate, casein and lipids (TWEEN 20, 40, 60 and 80) were examined according to the methods described by Tindall et al. (2007). Other physiological or biochemical tests were investigated by using the API 20E, API ZYM and API 50CHB Kits (all from BioMérieux, France) and Biolog Gen III microPlates according to the manufacturer’s instructions except that salinity was adjusted to 3%. All tests were carried out simultaneously with related type strains at least twice. Susceptibility to antibiotics was tested by measuring the size of the inhibition zone generated by a different drug-sensitive paper on MA at 28 °C for 3 days. A cell suspension (0.5 McFarland standard) was swabbed over MA to create a uniform lawn before aseptic placement of antibiotic discontos the surface. CLSI standards were strictly followed for cultivation and inhibition zone diameter reading (CLSI 2012).

**Chemotaxonomic characterization analysis**

To analyse the characterization of cellular fatty acids, respiratory quinone and polar lipid compositions, cells of strain E8T and two related type strains were harvested from MB medium after growth at 28 °C for 3 days and subjected to freeze-drying. The cellular fatty acids were saponified, methylated and extracted according to the standard protocols of MIDI (Sherlock Microbial Identification System, version 6.0B) protocol equipped with an Agilent HP6890 gas chromatograph (Sasser 1990). The designations and percentages of fatty acids were identified with the TSBA40 database using MIS standard software. The respiratory quinones were extracted from 300 mg freeze-dried cell material and separated by TLC after extraction with a chloroform/methanol (2:1, v/v) mixture (Tindall et al. 2007). The content of each quinone type was subsequently analyzed by HPLC according to the method previously described (Kroppenstedt 1982). The polar lipids were extracted according to the procedure described by Minnikin et al. (1984) and separated with solutions chloroform/methanol/water (65:25:4, by vol) for the first dimension and chloroform/methanol/acetic acid/water (80:12:15:4, by vol) for the second dimension via two-dimensional silica-gel TLC. The lipid material was stained using 10% molybdophosphoric acid solution (total lipids), molybdenum blue solution (phosphates), α-naphthol sulfuric solution (carbohydrates) and ninhydrin (amines), respectively. The specific experimental process is implemented according to the method described by Tindall et al. (2007).

**Comparative genome analysis and pan-genome analysis**

Comparative genomics of strain E8T and other species in the genus Marinomonas was performed using 29 reference genomes available in the GenBank database. The general features of the genomes of strains were obtained from the result of NCBI Prokaryotic Genome Annotation Pipeline (PGAP). To identify the genomic distance between each genomes, the MASH distance was computed by using MASH (Ondov et al. 2016). The pairwise whole genome comparisons of average nucleotide identity (ANI)
and average amino acid identity (AAI) were calculated according to Konstantinidis and Tiedje using the scripts (http://enve-omics.gatech.edu/). The digital DNA–DNA hybridization (dDDH) values were determined by using the Genome-to-Genome Distance Calculator (GGDC2.1) (http://ggdc.dsmz.de).

The protein sequences were clustered and compared by Cd-hit (Li and Godzik 2006) and aligned using MAFFT method (Katoh et al. 2002). The protein sequences were conserved into corresponding codon alignments and automated alignment trimming by using trimAl (Capella-Gutiérrez et al. 2009). Then, the single-copy protein tree was constructed by FastTree (Price et al. 2010). To identify conserved single-copy gene families, the genomes of strain E8T and 29 reference strains were analysed using OrthoFinder (Emms and Kelly 2019), following the default parameters and constructed a phylogenetic tree of single-copy Orthologue proteins by FastTree (Price et al. 2010). The pan-genome analysis was conducted by using BPGA (Bacterial Pan Genome Analysis tool) (Chaudhari et al. 2016) to identify the core, accessory and unique genes. The pipeline generated a phylogenetic tree based on pan-matrix data without outgroup used for the pan-genome tree for better evidence of the relationship among Marinomonas species. Furthermore, the OrthoVenn2 web server (Xu et al. 2019) was performed to analysed the protein sequences for comparison and annotation of the orthologous clusters of the strains E8T and 11 strains isolated from marine plant including M.agarivorans QM202T, M. algarazii CECT 7730T, M.algicola SM1966T, M.aquiplantarum CECT 7732T, M.balearica CECT 7378T, M.colpomeniae SM2066T, M.foliarum CECT 7731T, M.pollencensis CECT 8886T, M.pontica DSM 17793T, M.communis JCM 20766′, M.vulgaris A79T. All data are from this study unless otherwise indicated.

| Characteristic | 1 | 2 | 3 | 4* |
|---------------|---|---|---|----|
| Motile        | – | + | + | + |
| Growth at 35 °C | + | – | + | – |
| Growth in 10% NaCl | – | + | + | – |
| Hydrolysis of |   |   |   |   |
| Tween 20      |   | + | w | – |
| Gelatin       | – | + | – | – |
| Starch        | – | – | w | – |
| Sucrose       | – | + | – | – |
| Enzyme activities |   |   |   |   |
| Oxidase       | – | + | – | + |
| Catalase      | – | + | + | + |
| Valine arylamidase | + | w | w | + |
| α-glucosidase | – | + | + | + |
| Arginine dihydrolase | – | + | – | + |
| Oxidation of  |   |   |   |   |
| d-mannose     | + | – | + | – |
| d-mannitol    | + | w | + | + |
| d-maltose     | – | + | + | – |
| Fermentation of |   |   |   |   |
| d-sorbitol    | – | + | – | + |
| l-sorbose     | + | + | + | – |
| l-rhamnose    | – | w | + | – |
| Inositol      | – | + | + | – |
| d-turanose    | w | – | + | – |
| d-lyxose      | + | w | – | + |
| d-tagatose    | + | + | w | – |
| DNA G+C content (mol%) | 45.1 | 46.5 | 45.8 | 46.0 |

Results and discussion

Morphological, physiological and biochemical characteristics

Cells of the strain E8T were Gram-stain-negative, rod-shaped, about 0.2–0.4 µm wide and 1.0–2.5 µm long, without flagella (Fig. S1). A wide range of growth has been observed in temperature, pH and tolerance of salt, including strain E8T and related species in the genus Marinomonas. The major differences in biochemical characteristics for strain E8T and related species are summarized in Table 1. All negative traits of strain E8T are provided in Table S1. According to the result of API ZYM, strain E8T had the activity of alkaline phosphatase, esterase (C4), esterase

Table 1 Differential characteristics of strain E8T compared to its closely related species

- Positive, – negative, w weakly positive
- Data taken from Ying et al. (2021)
lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase. Cellulose, casein, starch, alginate, Tween 40, 60, 80 were not hydrolysed and negative for nitrate reduction in strain E8T. Strain E8T was found to be susceptible to (μg per disc) penicillin (10), streptomycin (10), tobramycin (10), gentamicin (10), ampicillin (10), ofloxacin (5), carbenicillin (100), ceftriaxone (30), norfloxacin (10), cefotaxime (30), clarithromycin (CLR) (15), polymyxin B (300), chloramphenicol (30), intermediate to neomycin (30), rifampicin (5), kanamycin (30), erythromycin (15), and resistant to vancomycin (VA) (30), tetracycline (30), lincomycin (2).

**Phylogenetic and phylogenomic analyses**

A nearly full-length 16S rRNA gene sequence (1425 bp) of strain E8T obtained from PCR amplification was included in the 16S rRNA gene sequences assembled from genomic sequences which contained only one complete 16 s rRNA. The BLAST search in NCBI revealed that strain E8T exhibited highest similarities with *M. vulgaris* A79T (98.4%) and *M. pontica* 46-16T (97.5%). The similarity between strain E8T and *Marinomonas communis* JCM 20766T, the type species of the genus *Marinomonas*, was 94.9%. As the topology is based on a maximum-likelihood phylogenetic tree of 16 s
rRNA genes, strain E8\textsuperscript{T} was clustered with \textit{M. vulgaris} A79\textsuperscript{T} at a bootstrap formed a separated branch (Fig. 1). The neighbour-joining and maximum parsimony trees also support this branch with high bootstrap values (Fig. S2 and Fig. S3). In addition, the phylogenetic tree based on the genomic sequences with IQ Tree showed the clade formed by strain E8\textsuperscript{T} and \textit{M. vulgaris} A79\textsuperscript{T} could distinguished from other members in the genus and confirming the strain E8\textsuperscript{T} was a member of the genus \textit{Marinomonas} (Fig. 2).

**General genomic features**

The sequence of the draft genome of strain E8\textsuperscript{T} was assembled into 79 contigs with a total length of 3,285,337 bp, a contig N50 value of 126,031 bp, a contig L50 value of 8 and a mean coverage of 150×. The longest contig and the shortest contig were 379,893 bp and 618 bp. The DNA G+C content was 45.1 mol%, which was in the middle of most of the related species. A total of 3085 genes were predicted with 2988 protein-coding genes and 97 encode RNAs including 8 5S rRNAs, 4 16S rRNAs, 4 23S rRNAs, 77 rRNAs and 4 ncRNAs. The genome sequence of strain E8\textsuperscript{T} included 31 Long interspersed nuclear elements (LINEs), 23 Short interspersed nuclear elements (SINEs), 4 Genomics Islands (GIs), 2 CRISPR-associated genes. Further comparative general features of 30 genome sequence were shown in Table S2.

According to the genomic functions predicted, the strain E8\textsuperscript{T} has been annotated with complete glycolysis, gluconeogenesis, citrate cycle (TCA cycle) and pentose phosphate pathway for central carbohydrate metabolism and UDP-N-acetyl-D-glucosamine biosynthesis, which was same as \textit{M. vulgaris} A79\textsuperscript{T} and \textit{M. communis} JCM 20766\textsuperscript{T}. The succinate dehydrogenase, cytochrome bd ubiquinol oxidase, cytochrome o ubiquinol oxidase and F-type ATPase were predicted in strain E8\textsuperscript{T} and \textit{M. vulgaris} A79\textsuperscript{T} but the lack of cytochrome c oxidase and cytochrome bc1 complex respiratory unit which presented in JCM 20766\textsuperscript{T} genome. The ackA gene was absented in strains E8\textsuperscript{T} and \textit{M. vulgaris} A79\textsuperscript{T} genomes which were incapable to convert acetyl-CoA into acetate as a carbon fixation pathway completed in JCM 20766\textsuperscript{T} genome. The sulfate reduction ability was predicted in \textit{M. vulgaris} A79\textsuperscript{T} with cysNC, cysN, cysD, cysNC, cysC, cysH, cysJ and cysI genes to convert sulfate into sulfide but the cysC gene was the absence in strain E8\textsuperscript{T} genome which may just convert sulfite into sulfide. The strain E8\textsuperscript{T} was predicted to metabolism many amino acids including serine, threonine, valine, isoleucine, leucine, lysine, ornithine, arginine, proline and tryptophan. For the metabolism of cofactors and vitamins, biosynthesis pathway of Pyridoxal-P (EC 1.4.3.5), NAD (EC 6.3.5.1), pantothenate (EC 6.3.2.1),...
coenzyme A (EC 6.3.2.5), biotin (EC 2.8.1.6), lipoic acid (EC 2.8.1.8), molybdenum cofactor (EC 2.10.1.1), siroheme (EC 4.99.1.4), heme (EC 1.3.5.3) and cobalamin (EC 6.3.1.10) were completed in strain E8T genome sequence. The genomes of three strains contain several genes responsible for choline uptake and betaine biosynthesis, including choline dehydrogenase (betA), betaine-aldehyde dehydrogenase (betB) and glycine betaine ABC transport system permease (ProU), as osmoprotectant. All genes for the synthesis of PG and PE have been found in the genome of strain E8T, which were similar to other strains in the genus Marinomonas.

**Chemotaxonomic characterisation**

The fatty acids profiles of strain E8T and reference strains M.pontica DSM 17793T and M.communis JCM 20766T were shown in Table S3. The major fatty acids (> 10%) were C16:0, summed feature 3 (C16:1ω7c and/or C16:1ω6c), and summed feature 8 (C18:1ω7c and/or C18:1ω6c). The summed feature 8 ratio with the feature of 47.8%, which was the most abundant cellular fatty acid of the strain E8T, and was also the most abundant fatty acid of the related type strains M.pontica DSM 17793T and M.communis JCM 20766T in the ratio of 35.3 and 45.7%. The strain E8T can be distinguished from other type strains in the compounds of C17:0 with the ratio of 3.1% which composition was less than 0.5% in other type strains. The composition of fatty acid summed features 8 in strain E8T was significantly higher than that in M.pontica DSM 17793T but similar to that in M.communis JCM 20766T. The polar lipids were mainly composed of phosphatidylglycerol (PG), phosphatidylethanolamine (PE) and three unidentified lipids (L1–L3), which were similar to other type strains with PG and PE as the major polar lipids (Fig. S4). The

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**Fig. 3** Pairwise comparisons of the average nucleotide identity (ANI) and average amino acid identity (AAI) between genome sequencing of strain E8T and reference genome sequences in genus Marinomonas. Upper triangle: ANI values; Lower triangle: AAI values.
isoprenoid quinone detected in strain E8T was consistent with the other related type strains, which was Q-8.

**Comparative genome analysis**

The result of the genomic distance between each genome sequence showed that strain E8T has the closest distance with *M. vulgaris* A79T and far distance with *M. agarivorans* QM202T, *M. flavescens* ANRC-JHZ47T and *M. posidonica* IVIA-Po-181T (Fig. S5). The ANI and AAI values between strain E8T and *M. vulgaris* A79T were 78.7 and 84.3%, which is below the recommended cut-off value of 95–96% (Richter and Rossello-Mora 2009) and below the proposed cut-off for a species boundary of 85–90% (Qin et al. 2014), respectively. Strain E8T also shared low dDDH values with *M. vulgaris* A79T (22.0%), below the 70% for species boundary (Goris et al. 2007; Meier-Kolthoff et al. 2013). Moreover, the pairwise comparisons of the digital DDH values were shown in Table S4 and the pairwise genome comparisons in nucleotide level and protein translated genes (ANI and AAI values) were shown in Fig. 3, which suggested that strains E8T represent a putative novel species of the genus *Marinomonas*.

The single copy core protein tree showed that strain E8T and *M. vulgaris* A79T shaped a branch with close distance with bole formed by all 30 reference strains (Fig. S6). The strains *M. agarivorans* QM202T and *M. algicola* SM1966T have a long branch length with bole which may portend rather distant phylogenetic relationships. The phylogenetic tree constructed by single-copy orthologue proteins also conformed to the tree (Fig. S6). According to the result of single-copy gene families, 6806 orthogroups with 104,049 genes in orthogroups and 3629 unassigned genes have been clustered and 1379 orthogroups which presented in all 30 species.

The pan-genome orthologous groups (POGs) of the 30 *Marinomonas* strains indicated that strain E8T had 959 core genes, 1661 accessory genes, 219 unique genes and 10 exclusively absent genes. The plot of core-pan showed that the pan-genome in *Marinomonas* can be considered as “open” (Fig. S7). The pipeline generated a pan-genome phylogenetic tree without outgroup based on pan-matrix data which highlighted distinct groups and confirmed in the core-genome tree (Fig. S8, S9). According to the KEGG annotation, the strains in the genus *Marinomonas* showed activity in amino acid metabolism, carbohydrate metabolism, membrane transport, metabolism of cofactors and vitamins and xenobiotics biodegradation and metabolism. Unique genes abundantly distributed among amino acid metabolism, carbohydrate metabolism and xenobiotics biodegradation (Fig. S10). The selected type strains (12 species) related to marine plant formed 5297 clusters, 3916 of which are orthologous clusters (at least contains two species) and 1381 of which are single-copy gene clusters (Table S5). Five genomes shared 1510 protein-coding regions and genome of strain E8T encodes 2904 proteins contained 2604 in clusters and 261 which found as singletons (Fig. 4).

The strain E8T with *M. aquimarina* CECT 5080T and *M. vulgaris* A79T have been observed with a low frequency of carbohydrate transport and metabolism (COG function category G) which may associate with their oligotrophic environment. Compare to strain A79T, strain E8T showed a relatively high count of proteins in category J, X and D which were related to genetic information transmission and transcription (Fig. 5). The proteins relevant to secondary metabolites biosynthesis were observed to present in all strains. According to the antiSMASH annotations, the abundant BGCs have been predicted in the genomic sequences of 30 strains. The genomes of the strain *M. mediterranea* MMB-1T, *M. spartinae* CECT 8886T, *M. posidonica* IVIA-Po-181T and *M. primoryensis* MPKMM3633T contained the highest bioclusters, as shown in Fig. 6. In general, the genomes of most of the strains were predicted to encode for ectoine, non-ribosomal polyketide synthetases (NRPS), betalactone and some other unspecified ribosomally synthesised and post-translationally modified peptide product.
(RiPP) cluster (Pipp-like). The Pipp-like in strain E8T has been annotated with bacteriocin which has been annotated in Marinomonas primoryensis MPKMM3633^T, Marinomonas pollencensis CECT 7375^T and Marinomonas spartinae CECT 8887 showing a high percentage genes similarity. The MiBiG comparison showed that the bacteriocin annotated in strain E8T had the highest similarity score with compound lanthipeptide (MiBiG accession number BGC0000054) from Streptomyces filamentosus NRRL 15,998. In addition, M. spartinae CECT 8887^T has been annotated with a Type I PKS (Polyketide synthase) genes cluster which may mediate the biosynthesis of cylindrospermopsin. According to the BAGEL4 prediction, lasso peptide was predicted in M. polaris DSM 16579^T, M. Mediterranea MMB-1^T and
M. rhizomae IVIA-Po-145\textsuperscript{T}; Microcin was predicted in both M. piezotolerans YLB-05\textsuperscript{T} and M. algicola SM1966\textsuperscript{T}; Butyrivibriocin, garvicin\_ML, bottromycin were predicted in M. rhizomae IVIA-Po-145\textsuperscript{T}, M. flavescens ANRC-JHZ47\textsuperscript{T} and M. atlantica Cmf 18.22\textsuperscript{T}, respectively.

**Taxonomic conclusion**

Phylogenetic analysis based on 16S rRNA sequence and genomic sequence showed that strain E8\textsuperscript{T} clustered with M. vulgaris A79\textsuperscript{T} belongs to the genus *Marinomonas*, which was consistent with chemotaxonomic results (Table S3). However, low levels of DNA relatedness shared with closely related species based on digital analyses (Fig. 3 and Table S4) and phenotypic characteristics (Table 1) could distinguished strain E8\textsuperscript{T} from other type strains in the genus *Marinomonas*. These results demonstrated that strain E8\textsuperscript{T} represent a novel species of the genus *Marinomonas*, for which the name *Marinomonas algarum* sp. nov. is proposed.

**Description of *Marinomonas algarum* sp. nov.**

*Marinomonas algarum* (al.g.a’rum. L. gen. pl. n. algarum of/from algae).

Cells are Gram-stain-negative, rod-shaped, non-flagellated, catalase- and oxidase-negative and strictly aerobic bacterium, approximately 0.2–0.4 \( \mu \)m wide and 1.0–2.5 \( \mu \)m long. The colonies are white, round and smooth after incubation at 28 \( ^\circ \)C on marine agar 2216 for 48 h. Growth occurs in presence of 0.5–8% NaCl (w/v; optimum 1–3\%), at a temperature of 15–35 \( ^\circ \)C (optimum 25–30 \( ^\circ \)C), and at pH 6.0–9.0 (optimum 6.5–8.0). Growth can be observed with \( \delta \)-mannose, \( \delta \)-mannitol, citrate and arabinose. Acid is produced from \( \delta \)-ribose, \( \delta \)-xylose, \( \lambda \)-xylose, \( \delta \)-fructose, \( \lambda \)-sorbose, esculin ferric citrate, \( \delta \)-lyxose, \( \delta \)-turanose, \( \delta \)-tagatose, potassium 5-ketogluconate. Tween 20 and gelatin are hydrolysed. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase are positive. Alginate, starch, casein, cellulose and Tween 40, 60 and 80 are not hydrolyzed and negative for nitrate reduction. The
predominant isoprenoid quinone is Q-8 and the major fatty acids are C_{16:0}, summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c) and summed feature 8 (C_{18:1}ω7c and/or C_{18:1}ω6c). The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine. The type strain is E8T (= KCTC 92201T = MCCC 1K07070T), isolated from red algae Gelidium amansii. The DNA G+C content of the type strain is 42.8 mol%.

The GenBank accession number for the 16S rRNA sequence is OK444097, and the draft genome sequence of strain E8T has been deposited at GenBank under accession number JAJTW000000000.

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Author contributions J-HX and B-NZ isolated the strain E8T, carried out the phylogenomic analysis, analysed most of the data. J-HX, B-NZ, W-JW, Z-MW, FZ, Y-YL, YS and P-XY performed the experiments. J-HX carried out a comparative genomic analysis. The initial draft of the paper was written by J-HX. L-HZ and XX conceived of the study, designed the study, critically revised the manuscript and co-corresponding the study. All authors read, discussed the results and revised the manuscript.

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Declarations

Conflict of interest Authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animal experiments by any of the authors.

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