Draft genome sequence of *Marinobacterium rhizophilum* CL-YJ9<sup>T</sup> (DSM 18822<sup>T</sup>), isolated from the rhizosphere of the coastal tidal-flat plant *Suaeda japonica*

Dong Han Choi<sup>1†</sup>, Gwang II Jang<sup>2†</sup>, Alla Lapidus<sup>3,4</sup>, Alex Copeland<sup>5</sup>, T. B. K. Reddy<sup>5</sup>, Supratim Mukherjee<sup>5</sup>, Marcel Huntemann<sup>6</sup>, Neha Varghese<sup>6</sup>, Natalia Ivanova<sup>3</sup>, Manoj Pillay<sup>6</sup>, Brian J. Tindall<sup>7</sup>, Markus Göker<sup>7</sup>, Tanja Woyke<sup>5</sup>, Hans-Peter Klenk<sup>8</sup>, Nikos C. Kyrpides<sup>5</sup> and Byung Cheol Cho<sup>2*</sup>

**Abstract**

The genus *Marinobacterium* belongs to the family *Alteromonadaceae* within the class Gammaproteobacteria and was reported in 1997. Currently the genus *Marinobacterium* contains 16 species. *Marinobacterium rhizophilum* CL-YJ9<sup>T</sup> was isolated from sediment associated with the roots of a plant growing in a tidal flat of Youngjong Island, Korea. The genome of the strain CL-YJ9<sup>T</sup> was sequenced through the Genomic Encyclopedia of Type Strains, Phase I: KMG project. Here we report the main features of the draft genome of the strain. The 5,364,574 bp long draft genome consists of 58 scaffolds with 4762 protein-coding and 91 RNA genes. Based on the genomic analyses, the strain seems to adapt to osmotic changes by intracellular production as well as extracellular uptake of compatible solutes, such as ectoine and betaine. In addition, the strain has a number of genes to defense against oxygen stresses such as reactive oxygen species and hypoxia.

**Keywords:** Genome, *Marinobacterium rhizophilum*, *Suaeda japonica*, Rhizosphere, GEBA

**Introduction**

The genus *Marinobacterium* within the family *Alteromonadaceae* was established in 1997 by González et al. [1]. Currently the genus *Marinobacterium* contains 16 species with validly published names (Fig. 1). All *Marinobacterium* strains have been isolated from marine environments [1–11] such as sea water, tidal flat, deep-sea sediment, and coral mucus. Interestingly, their habitats include tropical waters [12, 13], Arctic marine sediment [7], tidal flats [4, 11] as well as deep sea sediment [10], indicating that the genus has well adapted to diverse environments. In the GOLD database [14], genome sequencing of 38 strains from 11 *Marinobacterium* species are identified to be finished or in progress. In addition, six genome sequences from five species (*M. jannaschii*, *M. litorale*, *M. rhizophilum*, *M. stanieri* and *M. profundum*) and one unidentified strain are found in the GenBank database. Among them, genomic features of *M. rhizophilum* CL-YJ9<sup>T</sup> (=DSM 18822=KCCM 42386<sup>T</sup>), isolated from the rhizosphere of a plant *Suaeda japonica* inhabiting a coastal tidal flat, Korea, will be presented here.

**Organism information**

**Classification and features**

By phylogenetic analysis of the 16S rRNA gene sequence (Fig. 1), *M. rhizophilum* strain CL-YJ9<sup>T</sup> was positioned within the genus *Marinobacterium* and formed a distinct branch together with *Marinobacterium profundum* PAMC 27536<sup>T</sup> and *Marinobacterium nitratireducens* CN44<sup>T</sup> (Fig. 1). Strain CL-YJ9<sup>T</sup> was most closely related to *Marinobacterium profundum* PAMC 27536<sup>T</sup>, which appeared as its sister species in the tree. Strain CL-YJ9<sup>T</sup> grows under strictly aerobic conditions (Table 1). The optimal growth of strain CL-YJ9<sup>T</sup> occurs at pH 7.0, with a growth range of...
pH 6.0–9.0. Growth occurs in the presence of 1.0–5.0% (w/v) NaCl (optimum 3.0%) and at 5–30 °C (optimum 25 °C) (Table 1). Cells of strain CL-YJ9<sup>T</sup> are rod-shaped, on average approximately 0.3–0.4 μm wide and 0.6–0.8 μm long and motile by means of monopolar flagella (Fig. 2).

**Genome sequencing information**

**Genome project history**
The strain CL-YJ9<sup>T</sup> was chosen for genome sequencing by the phylogeny-based selection [15, 16] as a part of the Genomic Encyclopedia of Type Strains, Phase I: the KMG project [17]. The KMG project, the first of the production phases of the GEBA: sequencing a myriad of type strains initiative [18, 19] and a Genomic Standards Consortium project [20] was set up to increase the sequencing coverage of key reference microbial genomes and to generate a large genomic basis for the discovery of genes encoding novel enzymes [21]. The genome sequencing, finishing and annotation were performed by the DOE-JGI using state of the art sequencing technology [22]. A summary of the project information is presented in Table 2.

**Growth conditions and genomic DNA preparation**
*Marinobacterium rhizophilum* strain CL-YJ9<sup>T</sup> was grown in DSMZ medium 514 (http://www.dsmz.de) at 28 °C and aerobic conditions. Genomic DNA was isolated using Jetflex Genomic DNA Purification Kit (GENOMED 600100) following the standard protocol provided by the manufacturer but additionally applying 50 μl proteinase K and using a 60 min incubation time. DNA is available through the DNA Bank Network [23].

**Genome sequencing and assembly**
Using the purified genomic DNA, the draft genome of *M. rhizophilum* CL-YJ9<sup>T</sup> was generated at the DOE-JGI using the Illumina technology [24]. An Illumina standard shotgun library was constructed and sequenced using the Illumina HiSeq 2000 platform which generated 7,253,734 reads totaling 1088.1 Mbp. All general aspects of library construction and sequencing performed at the JGI can be found at the JGI website. All raw Illumina sequence data was passed through DUK, a filtering program developed at JGI, which removes known Illumina sequencing and library-preparation artifacts [25]. The
The following steps were then performed for assembly: (1) filtered Illumina reads were assembled using Velvet (version 1.1.04) [26], (2) 1–3 Kbp simulated paired end reads were created from Velvet contigs using wgsim (https://github.com/lh3/wgsim), (3) Illumina reads were assembled with simulated read pairs using Allpaths–LG (version r41043) [27]. Parameters for assembly steps were exactly same as in Choi et al. [28]. The final draft assembly contained 68 contigs in 58 scaffolds. The total size of the genome is 5.4 Mbp and the final assembly is based on 638.1 Mbp of Illumina data, which provides an average 119.1X coverage of the genome.

**Genome annotation**

As described in Choi et al. [28], identification of genes was performed using Prodigal [29] as part of the DOE-JGI Annotation pipeline [30, 31]. After translation of the

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**Table 1** Classification and general features of *M. rhizophilum* CL-YJ9T [8, 9]

| MIGS ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
| MIGS-6  | Domain   | Bacteria | TAS [39]     |
|         | Phylum   | Proteobacteria | TAS [40] |
|         | Class    | Gammaproteobacteria | TAS [41] |
|         | Order    | Alteromonadales | TAS [42] |
|         | Family   | Alteromonadaceae | TAS [43] |
|         | Genus    | Marinobacterium | TAS [1] |
|         | Species  | Marinobacterium rhizophilum | TAS [4] |
|         |          | Type strain CL-YJ9T | TAS [4] |
|         | Gram stain | Negative | TAS [4] |
|         | Cell shape | Straight rods | TAS [4] |
|         | Motility | Motile | TAS [4] |
|         | Sporulation | Not reported |NAS |
|         | Temperature range | 5–30 °C | TAS [4] |
|         | Optimum temperature | 25 °C | TAS [4] |
|         | pH range; Optimum | 6.0–9.0, 7.0 | TAS [4] |
|         | Carbon source | Glucose, sucrose, mannose, glycerol, glycine, mannitol | TAS [4] |
| MIGS-6.3 | Salinity | 1–5% (optimum: 3%) | TAS [4] |
| MIGS-22 | Oxygen requirement | Strictly aerobic | TAS [4] |
| MIGS-15 | Biotic relationship | Microbiota of the rhizome of Suaeda japonica | TAS [4] |
| MIGS-14 | Pathogenicity | Non-pathogenic | NAS |
| MIGS-4  | Geographic location | Youngjong Island, Korea | TAS [4] |
| MIGS-5  | Sample collection | November, 2005 | TAS [4] |
| MIGS-4.1 | Latitude | 37.485° N | TAS [4] |
| MIGS-4.2 | Longitude | 126.516° E | TAS [4] |
| MIGS-4.3 | Depth | Not reported | NAS |
| MIGS-4.4 | Altitude | Not reported | NAS |

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*Evidence codes - IDA inferred from direct assay, TAS traceable author statement (i.e., a direct report exists in the literature), NAS non-traceable author statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from the Gene Ontology project [44].

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**Table 2** Genome sequencing project information

| MIGS ID | Property | Term |
|---------|----------|------|
| MIGS-31 | Finishing quality | Level 1: Standard Draft |
| MIGS-28 | Libraries used | Illumina Std shotgun library |
| MIGS-29 | Sequencing platforms | Illumina HiSeq 2000 |
| MIGS-31.2 | Fold coverage | 119.1X |
| MIGS-30 | Assemblers | Velvet v. 1.1.04, ALLPATHS v. R37654 |
| MIGS-32 | Gene calling method | Prodigal v2.5 |
|         | Locus Tag | F451 |
|         | Genbank ID | ARUM00000000 |
|         | Genbank Date of Release | 12-Dec-2013 |
|         | GOLD ID | Gp0013985 |
|         | BIOPROJECT | PRJNA181367 |
| MIGS-13 | Source Material Identifier | CL-YJ9 |
|         | Project relevance | GEBA-KMG, Tree of Life |
predicted CDSs, they were used to search the databases, such as National Center for Biotechnology Information non-redundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional analysis and functional annotation were performed within the Integrated Microbial Genomes [32].

**Genome properties**
The genome is 5,364,574 bp long and comprises 58 scaffolds ranging 1097 to 401,958 bp, with an overall G + C content of 58.5% (Table 3). Of the 4853 genes predicted, 4762 were protein coding genes, and 91 were RNA genes. A total of 3878 genes (79.9%) were assigned a putative function while the remaining ones were annotated as hypothetical or unknown proteins. The distribution of genes into COG functional categories is presented in Table 4. The properties and the statistics of the genome are summarized in Tables 3 and 4.

**Insights from the genome sequence**
To cope with osmotically varying conditions in tidal flat (e.g., exposure to heavy rainfalls or desiccation during low tides), *M. rhizophilum* CL-YJ9T seems to display diverse mechanisms of adaption. For instance, the strain can synthesize compatible solutes such as betaine, ectoine and 5-hydroxyectoine. The strain has two kinds of genes (choline dehydrogenase and betaine aldehyde dehydrogenase; Table 5) participating in glycine-betaine biosynthesis from choline, which is found in Gram-negative bacteria [33]. The strain also has essential genes participating in the ectoine biosynthesis and the 5-hydroxyectoine biosynthesis (five enzymes for the steps from aspartate to ectoine as well as 5-hydroxyectoine hydroxylase, respectively; Table 5) [34]. In addition, the strain seems to uptake osmolytes by transport from the external environment. In the genomic analysis, the glycine betaine/L-proline ABC transporter system known as proU, which is an operon that encodes a high-affinity ABC transporter system consisting of three proteins (ProV, ProW and ProX; F451DRAFT_00884, F451DRAFT_00885, F451DRAFT_00886, respectively) is found in the strain. Further, the homologue of the TRAP transporter (F451DRAFT_00922) involved in transport of external ectoine and hydroxyectoine is found in *M. rhizophilum*. Function of the TRAP transporter is elucidated in both *Halomonas elongata* DSM 2581 [35] and *Silicibacter pomeroyi* DSS-3 [36]. Ectoine/5-hydroxyectoine-binding periplasmic protein in *M. rhizophilum* showed amino acids sequence similarity of 35.1% and 33.8% with those of *H. elongata* (TeaA) and *S. pomeroyi* (UehA), respectively. The transported ectoine is used as the sole carbon and nitrogen source.

| Table 3 | Genome statistics |
|---------|-------------------|
| Attribute | Number | % of totala |
| Genome size (bp) | 5,364,574 | 100 |
| DNA coding (bp) | 4,619,007 | 86.10 |
| DNA G + C (bp) | 3,136,815 | 58.47 |
| DNA scaffolds | 58 | 100 |
| Total genes | 4853 | 100 |
| Protein coding genes | 4762 | 98.12 |
| RNA genes | 91 | 1.88 |
| Pseudo genes | 0 | |
| Genes in internal clusters | 642 | 13.23 |
| Genes with functional prediction | 3878 | 79.91 |
| Genes assigned to COGs | 3433 | 70.74 |
| Genes with Pfam domains | 4066 | 83.78 |
| Genes with signal peptides | 386 | 7.95 |
| Genes with transmembrane helices | 1137 | 23.43 |
| CRISPR repeats | 1 | |

The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

| Table 4 | Number of genes associated with general COG functional categories |
|---------|-------------------------|
| Code | Value | % of total | Description |
| J | 232 | 6.01 | Translation, ribosomal structure and biogenesis |
| A | 1 | 0.03 | RNA processing and modification |
| K | 289 | 7.48 | Transcription |
| L | 103 | 2.67 | Replication, recombination and repair |
| B | 2 | 0.05 | Chromatin structure and dynamics |
| D | 41 | 1.06 | Cell cycle control, cell division, chromosome partitioning |
| V | 72 | 1.86 | Defense mechanisms |
| T | 182 | 4.71 | Signal transduction mechanisms |
| M | 213 | 5.52 | Cell wall/membrane/envelope biogenesis |
| N | 71 | 1.84 | Cell motility |
| U | 58 | 1.50 | Intracellular trafficking, secretion, and vesicular transport |
| O | 162 | 4.19 | Post-translational modification, protein turnover, chaperones |
| C | 296 | 7.66 | Energy production and conversion |
| G | 334 | 8.65 | Carbohydrate transport and metabolism |
| E | 407 | 10.54 | Amino acid transport and metabolism |
| F | 102 | 2.64 | Nucleotide transport and metabolism |
| H | 211 | 5.46 | Coenzyme transport and metabolism |
| I | 179 | 4.63 | Lipid transport and metabolism |
| P | 186 | 4.82 | Inorganic ion transport and metabolism |
| Q | 134 | 3.47 | Secondary metabolites biosynthesis, transport and catabolism |
| R | 335 | 8.67 | General function prediction only |
| S | 209 | 5.41 | Function unknown |
| – | 1420 | 29.26 | Not in COGs |

The total is based on total number of protein coding genes in the annotated genome.
source in S. pomeroyi, but H. elongata can use it as a compatible solute. Considering that ectoine can be de novo produced in M. rhizophilum as well as actively transported from the environment, the role of the TRAP transporter in M. rhizophilum could be thought to recover endogenously synthesized ectoine that has leaked through the membrane as known in H. elongata [35].

In the rhizosphere of tidal flat, oxygen tension varies in a wide range due to temperature change, repetitive exposure to atmosphere and seawater during tidal cycle and oxygen release from the roots of plants. Further, M. rhizophilum has a molybdopterin biosynthesis pathway (Table 5) and molybdoenzymes that use molydopterin as a cofactor or prosthetic group such as formate dehydrogenase (F451DRAFT_01667, F451DRAFT_01668, F451DRAFT_01669, F451DRAFT_01665) involved in protection from oxidative stress and enhanced respiration under hypoxic conditions are present, indicating M. rhizophilum is adapted to the hypoxic rhizosphere in tidal-flat sediments, too.

The presence of motility by means of monopolar flagella was reported in a previous report [4]. Consistently, a number of genes encoding flagellar basal body proteins, flagellar hook-associated proteins and flagellar biosynthesis proteins are found in the genomic analyses, suggesting that M. rhizophilum could explore more favorable microenvironments using flagella in the rhizosphere. In contrast to a recent study that genes encoding steroid catabolism were identified in Marinobacterium stanieri S30 [38], most of these genes were not identified in the M. rhizophilum.

Conclusions

The genome of a representative of the genus Marinobacterium from the Proteobacteria phylum is reported here for the first time. In addition to detailed information on genome sequencing and annotation, genetic adaptation in environmental conditions closely associated with rhizosphere of a tidal flat plant such as salinity change and oxygen stress could be understood on the basis of genomic analyses.

Abbreviations

GBEA: Genomic Encyclopedia of Bacteria and Archaea; GOLD: Genomes OnLine Database; JGI: Joint Genome Institute; KMG: One thousand microbial genomes; ROS: Reactive oxygen species; TRAP: Tripartite ATP-independent periplasmic

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Authors’ contributions

DHC, GJ and BCC drafted the manuscript. AL, AC, TBKR, SM, MH, NV, NL, MP, BJT and TW sequenced, assembled and annotated the genome. NCK, MG, HPK designed the KMG study and selected the strain for sequencing. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests. Authors Kyrpides and Klenk are editorial board members of the Standards in Genomic Sciences.

| Pathways | Enzymes | Gene-loci |
|----------|---------|-----------|
| Glycine betaine biosynthesis | Choline dehydrogenase | F451DRAFT_01661, F451DRAFT_03441, F451DRAFT_04658 |
| Ectoine and 5-hydroxyectoine biosynthesis | Aspartate kinase | F451DRAFT_00077, F451DRAFT_02577 |
| Molybdopterin biosynthesis | Cyclic pyranopterin monophosphate synthase | F451DRAFT_03412, F451DRAFT_01249 |

Table 5 Enzymes and gene-loci participating in selected pathways identified in the draft genome of M. rhizophilum CL-YJ9T. Gene-loci are from the IMG/MER database.
Marinobacterium lutimaris

Marinobacterium aestuariivivens

Marinobacterium litorale

Marinobacterium coralli
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