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Data

Complete genome sequence of *Rhodoferax* sp. PAMC 29310 from a marine sediment of the East Siberian Sea

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ABSTRACT

Rhodoferax sp. PAMC 29310 was isolated from a surface marine sediment of the East Siberian Sea, Arctic. Whole-genome sequencing of the strain *Rhodoferax* sp. PAMC 29310 was achieved using PacBio RS II and Illumina platform. The resulting complete genome comprised of 4,593,249 base pairs (G + C content of 58.0%) with a single chromosome, 4546 protein-coding genes, 57 tRNAs and 6 rRNA operons. A complete set of genes encoding the enzymes of glycolysis and citric acid cycle were identified. No genes encoding ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) and nitrogenase reductase (*nif*) were present indicating that strain PAMC 29310 is not capable of fixing of carbon and nitrogen. PAMC 29310 genome contains genes for dissimilatory and assimilatory nitrate reduction. Gene encoding choline dehydrogenase enzyme which functions at the first step in the synthesis of betaine, one of the most effective osmoprotectants, was detected. In particular, among the genomes of the genus *Rhodoferax* strains, gene encoding nitrite reductase (*nir*K), which reduces nitrite to nitric oxide and *tetA* gene encoding tetracycline resistance protein involved in the resistance to tetracycline were identified only in the genome of *Rhodoferax* sp. PAMC 29310. As the first genome from the strain which was isolated from marine sediment in the genus *Rhodoferax*, investigation of physiological characteristics based on the complete genome sequences will help understand the adaptation of *Rhodoferax* sp. PAMC 29310 in the marine sediment.

1. Introduction

The genus *Rhodoferax* was first proposed with purple non-sulfur bacteria *Rhodoferax fermentans* (Hiraishi et al., 1991) and it belongs to the family *Comamonadaceae* within the phylum *Proteobacteria*. At the time of writing, 9 type strains of the genus *Rhodoferax, R. antarcticus, R. aquaticus, R. bucti, R. fermentans, R. ferrireducens, R. koreense, R. lacus, R. saidenbachensis,* and *R. sediminis* isolated from an algal-bacterial mat, freshwater, sewage, sludge, and freshwater sediment are known with G + C contents in the range of 57.4%–65.6% (Farh et al., 2017; Finneran et al., 2003; Hiraishi et al., 1991; Jin et al., 2020; Kaden et al., 2014; Li et al., 2020; Madigan et al., 2000; Park et al., 2019; Zhou et al., 2019). Strains of the genus *Rhodoferax* are mesophilic or psychrotolerant species with a growth temperature range between 0 and 37 °C (with an optimum growth temperature range of 15–30 °C) (Farh et al., 2017; Finneran et al., 2003; Hiraishi et al., 1991; Jin et al., 2020; Kaden et al., 2014; Li et al., 2020; Madigan et al., 2000; Park et al., 2019; Zhou et al., 2019). Members of the genus *Rhodoferax* except for *R. antarcticus* were isolated from non-saline samples and grew at 0–1% of NaCl. Although *R. antarcticus* was isolated from an algal-bacterial mat collected from Antarctic ponds with approximately seawater salinity, this strain was rather sensitive to NaCl showing slow growth at 1% of NaCl (Madigan et al., 2000). *Rhodoferax* is well known for its diverse metabolic pathways. Two species, *R. fermentans* and *R. antarcticus*, grow photoautotrophically using hydrogen and carbon dioxide and photoheterotrophically using a variety of organic and fatty acids or glucose and are capable of nitrogen fixation (Hiraishi et al., 1991; Madigan et al., 2000) while remaining other strains are non-phototrophic and are capable of anaerobic dark fermentation, aerobic respiration and anaerobic growth via sugar fermentation (Farh et al., 2017; Finneran et al., 2003; Jin et al., 2020; Kaden et al., 2014; Li et al., 2020; Park et al., 2019; Zhou et al., 2019).

Rhodoferax sp. PAMC 29310 was isolated from a surface sediment collected from the East Siberian Sea and this is the first isolate of the genus *Rhodoferax* which was isolated from the marine habitat. Although salinity measured from the porewater of this sediment was 32.16 psu

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(practical salinity unit), *Rhodoferax* sp. PAMC 29310 did not require NaCl for growth and growth was inhibitory above 1% of NaCl. Genomes of all type strains of the genus *Rhodoferax* have been sequenced (Aurass and Flieger, 2020; Baker et al., 2017; Farh et al., 2017; Finneran et al., 2003; Hiraishi et al., 1991; Jin et al., 2020; Kaden et al., 2014; Li et al., 2020; Madigan et al., 2000; Park et al., 2019; Zhou et al., 2019). Here, we performed genome sequencing and present the complete genome sequence of the strain *Rhodoferax* sp. PAMC 29310.

2. Data description

Rhodoferax sp. PAMC 29310 was isolated by cultivation of a surface sediment collected from the East Siberian Sea using a multi-corer on 0.1× Marine agar (MA) (Difco, USA) plate at 10 °C for 30 days. Genomic DNA was extracted using a MG Genomic DNA Purification kit (MGmed, Korea) according to the manufacturer's instructions and sequencing was performed using the PacBio RS II (Pacific Biosciences, USA) and the Illumina HiSeq platform at Macrogen (Korea). Total 55,480 long-reads containing 547,936,161 bases and 10,510,210 short-reads containing 1,061,531,210 bases were generated, respectively. Long-reads were assembled into contigs using Hierarchical Genome Assembly Process 3 (HGAP3) within PacBio SMRT analysis 2.3 (Chin et al., 2013) and the resulting contigs were polished with short-reads using Pilon (v1.21) (Walker et al., 2014). Genome annotation was performed using the Rapid Annotation using Subsystems Technology (RAST) server (Aziz et al., 2008). Clusters of orthologous groups of proteins (COGs) were predicted by Prokka (version 1.12b) (Seemann, 2014) and metabolic pathways were predicted by using KEGG Automatic Annotation Server (KAAS) (Moriya et al., 2005).

The resulting complete genome comprised of 4,593,249 nucleotides with 58.0% G + C content (Table 1). No plasmid was found. 16S rRNA gene sequences retrieved from complete genome of strain PAMC 29310 showed 98.4% similarity with *Rhodoferax aquaticus* and *R. lacus* followed by *R. ferrireducens* (98.3%), *R. saidenbachensis* (98.3%), *R. antarcticus* (98.1%), *R. bucti* (97.9%), *R. fermentans* (97.9%), and *R. koreense* (97.9%). Average nucleotide identity (ANI) values among genomes of the genus *Rhodoferax* calculated by OrthoANIu algorithm (Yoon et al., 2017) were <74.9% and this level is below the ANI cut-off values (95–96%) to delineate bacterial species (Richter and Rosselló-Móra, 2009) indicating strain PAMC 29310 is a potentially novel species.

The predicted protein-coding sequences on the chromosome were 4546. The genome contains 57 tRNA genes and 6 rRNA operons (Table 1). Among the 4546 genes, 3235 genes (71.2%) were assigned to a subsystem using the SEED method (Overbeek et al., 2014) and 2092 genes to KEGG pathway. The function of 2095 genes was categorized by comparison with the COGs and the graphic circular map of the genome was generated using Circos map (Krzywinski et al., 2009) (Fig. 1).

Genome of strain *Rhodoferax* sp. PAMC 29310 contained genes of complete sets of glycolysis and the citric acid cycle. Genes encoding choline dehydrogenase enzyme, which functions at the first step in the synthesis of glycine betaine from choline was detected. Genes for the transport of glycine betaine, one of the most effective osmoprotectants, were also identified (Kappes et al., 1996). However, despite the presence of the genes involved in the osmotic tolerance, *Rhodoferax* sp. PAMC 29310 did not grow above 1% of NaCl (Table 1) indicating the necessity to examine the activity of this enzyme. PAMC 29310 genome contained genes for dissimilatory and assimilatory nitrate reduction.

Unlike two phototrophic species in the genus *Rhodoferax*, *R. fermentans* and *R. antarcticus,* genes encoding ribulose 1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which carboxylates ribulose 1,5-bisphosphate in the first step of the Calvin cycle to produce two molecules of 3-phosphoglycerate, were not found in the genome of strain PAMC 29310 indicating that strain PAMC 29310 is not capable of fixing carbon dioxide. Gene for nitrogenase reductase (*nif*) was not identified indicating that strain PAMC 29310 is not able to fix nitrogen.

Table 1
General features of *Rhodoferax* sp. PAMC 29310 and MIGS mandatory information.

Items	Description
General feature	
Current classification	Domain *Bacteria* Phylum *Proteobacteria* Class *Betaproteobacteria* Order *Burkholderiales* Family *Comamonadaceae* Genus *Rhodoferax* Species sp. Strain PAMC 29310
Gram-staining	Negative
Cell shape	Rod*
Motility	Non-motile
Temperature	4–20 °C (optimum, 15 °C)
Salinity	0–1% (optimum, 0–1%)
pH range	6.5–8.0 (optimum, 7.5)
MIGS data	
Submitted to insdc	CP072852
Investigation type	Bacteria
Geographic location	Arctic: East Siberian Sea
Latitude and longitude	73° 41′45.96″ N, 167° 40′32.46″ E
Collection date	2019-09
Environment (biome)	Marine biome [ENVO:00000447]
Environment (feature)	Cold environment [ENVO: 01000309]
Environment (material)	Marine sediment [ENVO: 03000033]
Relationship to oxygen	Facultatively aerobic
Sequencing platform	PacBio RS II with P6-C4 chemistry and Illumina
Fold coverage	102 x
Assembler	HGAP.3 and Pilon (v1.21)
Genome features	
Genome size (bps)	4,593,249
GC content (%)	58.0
CDSs in RAST	4546
CDSs assigned to subsystem in RAST	3235
CDSs assigned to COGs	2095
CDSs assigned to KEGG	2092
Hypothetical protein in RAST	1311
rRNA operons	6
tRNA genes	57

* Morphology of cells was examined by transmission electron microscopy and represented in supplementary Fig. S1.

Among the genomes of the genus *Rhodoferax* strains, gene encoding nitrite reductase (*nir*K) that reduces nitrite to nitric oxide was identified only in the genome of *Rhodoferax* sp. PAMC 29310. In addition, *tetA* gene encoding tetracycline resistance protein involved in the resistance to tetracycline was identified only in the genome of *Rhodoferax* sp. PAMC 29310. Genome of PAMC 29310 harbored genes involved in the biosynthesis and export of capsular polysaccharide (Table S1). A number of possible functions such as prevention of desiccation, adherence, and resistance to (non)specific host immunity of capsular polysaccharide have been known (Angelaalincy et al., 2018; Roberts, 1996) and validation on the functions of these genes in the *Rhodoferax* sp. PAMC 29310 is necessary. As the first genome from the strain that was isolated from marine sediment in the genus *Rhodoferax*, further investigation on physiological characteristics including genomic features that are found only in the genome of *Rhodoferax* sp. PAMC 29310 will provide information for better understanding on the adaptation of this strain in the marine environments.

Nucleotide sequence accession numbers

The complete genome sequence of *Rhodoferax* sp. PAMC 29310 has been deposited at GenBank under the accession number CP072852. This strain is available from Polar and Alpine Microbial Collection (PAMC) with the accession number PAMC 29310.

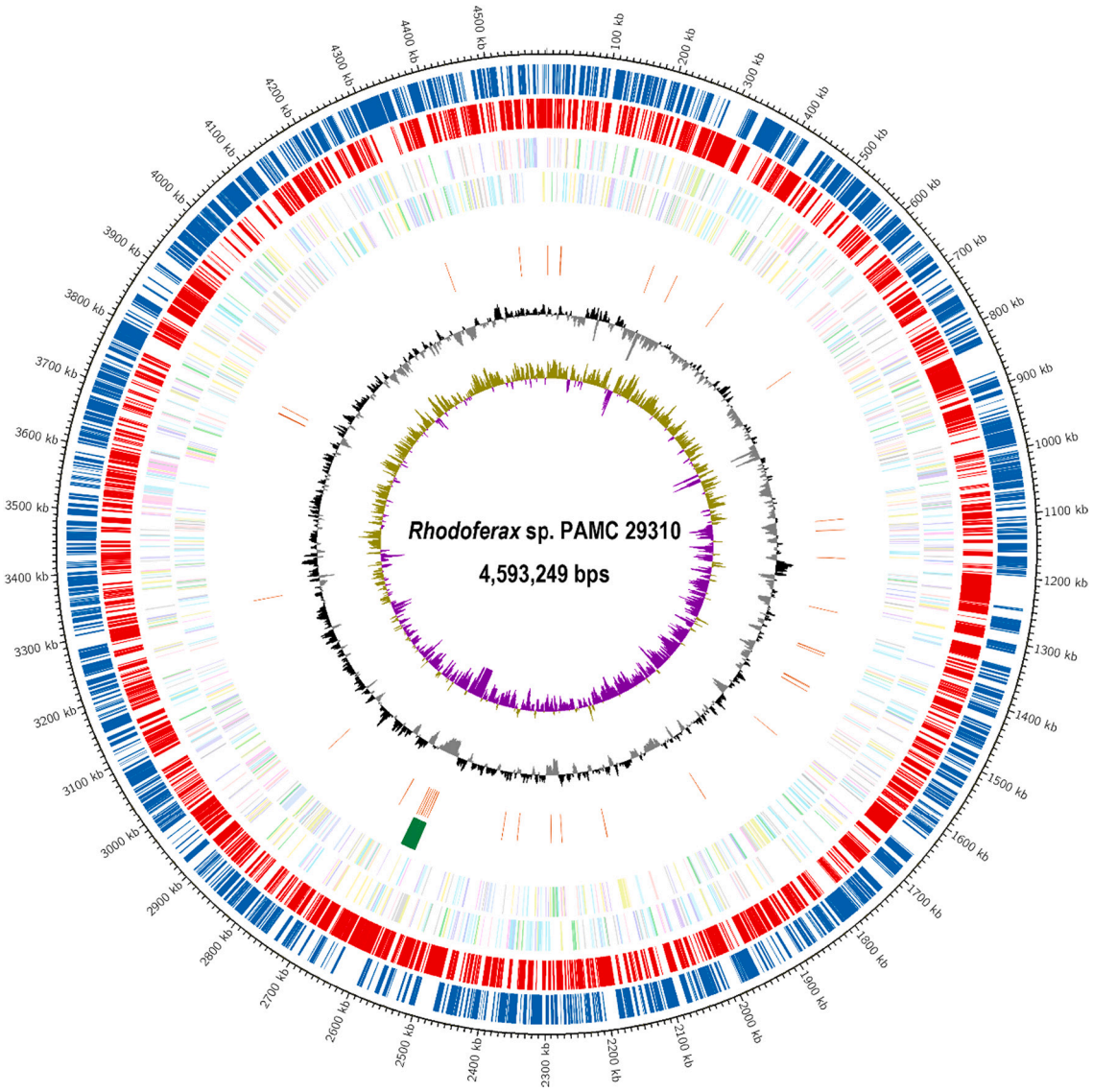

Fig. 1. Circular map of the *Rhodoferax* sp. PAMC 29310 genome. Labeling from outside to the center: genes on forward strand, genes on reverse strand, RNA genes (rRNAs green, tRNAs red), GC content (black/grey), and GC skew (olive/purple). Individual genes are colored by COG categories: J (translation, ribosomal structure, and biogenesis), A (RNA processing and modification), K (transcription), L (replication, recombination, and repair), B (chromatin structure and dynamics), D (cell cycle control, cell division, and chromosome partitioning), Y (nuclear structure), V (defense mechanisms), T (signal transduction mechanisms), M (cell wall/membrane/envelop biogenesis), N (cell motility), Z (cytoskeleton), W (extracellular structures), U (intracellular trafficking, secretion, and vesicular transport), O (posttranslational modification, protein turnover, and chaperones), X (mobilome: prophages and transposons), C (energy production and conversion), G (carbohydrate transport and metabolism), E (amino acid transport and metabolism), F (nucleotide transport and metabolism), H (coenzyme transport and metabolism), I (lipid transport and metabolism), P (inorganic ion transport and metabolism), Q (secondary metabolites biosynthesis, transport, and catabolism), R (general functional prediction only), and S (function unknown). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Declaration of Competing Interest

There are no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.margen.2021.100891.

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