Draft genome sequence of *Rubidibacter lacunae* strain KORDI 51-2\(^T\), a cyanobacterium isolated from seawater of Chuuk lagoon

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A photoautotrophic cyanobacterium, *Rubidibacter lacunae* was reported in 2008 for the first time. The type strain, KORDI 51-2\(^T\), was isolated from seawater of Chuuk lagoon located in a tropical area. Although it belonged to a clade exclusively comprised of extremely halotolerant strains by phylogenetic analyses, *R. lacunae* is known to be incapable of growth at high salt concentration over 10%. Here we report the main features of the genome of *R. lacunae* strain KORDI 51-2\(^T\). The genome of *R. lacunae* contains a gene cluster for phosphonate utilization encoding three transporters, one regulator and eight C-P lyase subunits.

**Introduction**

*Rubidibacter lacunae* type strain KORDI 51-2\(^T\) (=KCTC 40015\(^T\)=UTEX L2944\(^T\)) is a photoautotrophic cyanobacterium isolated from lagoon seawater of Chuuk, Micronesia [1]. At this time, the genus *Rubidibacter* is comprised of a single isolate. Further, only three environmental 16S rRNA gene sequences in the NCBI showed relatively high sequence similarity of ca. 96% to 16S rRNA gene of the strain. Thus, the genus seems either to be a numerically rare cyanobacterium or, to exploit specific environments such as microbial mats. Actually, the most similar sequences (accession no. of DQ861063 and DQ861117 in GenBank) to *Rubidibacter* were obtained in microbial mats of a coastal hypersaline pool. Nonetheless, the strain KORDI 51-2\(^T\) is a non-extreme halotolerant member in the *Halothece* cluster, exclusively composed of extremely halophilic/halotolerant bacteria. Considering this contrasting phenotypic trait, genomic information of KORDI 51-2\(^T\) could provide a good clue to understand genomic adaptation of cyanobacteria at extreme salt condition. Here we present a summary of the genomic features of *R. lacunae* strain KORDI 51-2\(^T\).

**Classification and features**

By phylogenetic analysis of 16S ribosomal RNA genes (Figure 1), *R. lacunae* KORDI 51-2\(^T\) was clustered into the *Halothece* cluster. Four *Euhalothece* strains belonging to the cluster were isolated from a hypersaline pond (strains MPI 96N303 and MPI 96N304) or a solar evaporation pond (strains MPI95AH10 and MPI95AH13) in Mexico [2]. These strains showed sustained growth between 6-16% salinity, and several strains could grow even in NaCl saturated brine, suggesting that they are at least extremely halotolerant cyanobacteria [2]. *Dactylococcopsis salina* and other *Halothece* strains belonging to the cluster were also isolated from various hypersaline environments, such as a solar lake in Egypt, a solar evaporation pond in Spain and hypersaline lagoon in Australia [2,3]. On the contrary, *R. lacunae* KORDI 51-2\(^T\) was isolated from natural seawater and able to grow at a salinity between 2 and 7% (Table 1). In addition, *R. lacunae* KORDI 51-2\(^T\) contains phycoerythrin, which differentiated it from the other strains belonging to the ‘*Halothece*’ cluster [1]. The epifluorescence micrograph of the cells and other classification and general features were shown in Figure 2 and Table 1, respectively.
Figure 1. Neighbor-joining tree showing the phylogenetic position of *Rubidibacter lacunae* KORDI 51-2T relative to other close cyanobacterial strains. GenBank accession numbers for each strain are shown in parenthesis. The tree uses the Jukes-Cantor corrected distance model to construct a distance matrix. Bootstrap values above 60%, based on 1,000 resamplings, are shown at the branching points. Strains with genome sequence are underlined.

Figure 2. Epifluorescence micrograph of *R. lacunae* KORDI 51-2T. The picture was taken under green excitation and then converted to gray scale. Bar, 3 μm.
Table 1. Classification and general features of *R. lacunae* strain KORDI 51-2T according to the MIGS recommendations [4]

| MIGS ID | Property          | Term                      | Evidence codea |
|---------|-------------------|---------------------------|----------------|
|         | Domain            | *Bacteria*                | TAS [5]        |
|         | Phylum            | *Cyanobacteria*           | TAS [6-8]      |
|         | Class             | *Cyanobacteria*           | TAS [8,9]      |
| Current classification | Order Unknown |                          |                |
|         | Family            | 1.1                       | TAS [7]        |
|         | Genus             | *Rubidibacter*            | TAS [1]        |
|         | Species           | *Rubidibacter lacunae*    | TAS [1]        |
|         | Type strain       | KORDI 51-2T               | TAS [1]        |
| Gram stain |                  | Not reported              |                |
| Cell shape |                  | Rods                      | TAS [1]        |
| Motility |                  | None                      | TAS [1]        |
| Sporulation |               | None                      | IDA           |
| Temperature range |            | 25-35°C                   | TAS [1]        |
| Optimum temperature |            | 30°C                      | TAS [1]        |
| MIGS-6  | Habitat           | Seawater                  | TAS [1]        |
| MIGS-6.3 | Salinity         | 2-7% (optimum: 5)         | TAS [1]        |
| MIGS-22 | Oxygen            | Aerobic                   | TAS [1]        |
|         | Carbon source     | Autotroph                 | TAS [1]        |
|         | Energy source     | Phototroph                | TAS [1]        |
| MIGS-15 | Biotic relationship | Free living              | TAS [1]        |
| MIGS-14 | Pathogenicity     | None                      | NAS           |
| MIGS-4  | Geographic location | Chuuk state, Micronesia  | TAS [1]        |
| MIGS-5  | Sample collection time | July, 2004               | IDA           |
| MIGS-4.1 | Latitude         | 7° 13’ N                  | IDA           |
| MIGS-4.2 | Longitude        | 151° 58’ E                | IDA           |
| MIGS-4.3 | Depth            | 40 m                      | IDA           |
| MIGS-4.4 | Altitude         | Not applicable            | NAS           |

a) 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 [9].
**Genome sequencing and annotation**

**Genome project history**

The organism was selected for sequencing on the basis of its phylogenetic position. The genome project was deposited in the Genomes On Line Database [10] and draft genome sequence was deposited in GenBank database (accession number ASSJ00000000). The genome sequencing was carried out in Macrogen Inc. (Seoul, Korea) using GS-FLX Titanium sequencing technology. Table 2 presents the project information and its association with MIGS version 2.0 compliance [4].

**Growth conditions and DNA isolation**

*R. lacunae* KORDI 51-2T was grown in a 50 ml culture flask filled with 50 ml of modified f/2 medium in which silicate was omitted and ammonium chloride was supplemented (final conc. of 100 μM). The culture flask with inoculum was incubated at 25°C at about 20 μE m² s⁻¹ (light:dark=14:10) for 3 weeks. Genomic DNA was isolated using Qiagen Genomic-tip 100/G (Qiagen) according to the manufacturer’s instruction.

**Genome sequencing and assembly**

The genome was sequenced by pyrosequencing (GS-FLX Titanium). A shotgun library was constructed according to GS FLX Titanium Sequencing Method Manual. The 291,414 pyrosequencing reads obtained has an average length of 442.12 bp and were assembled using the Newbler assembler (version, 2.3; Roche) with default options. The final assembly resulted in 126 contigs longer than or equal to 500 bp with the contigs sum of 4,215,105 bp. After removing 27 short contigs with low coverage in order to minimize possible contamination, the remaining 99 contigs were used for further analyses (Table 3).

**Genome annotation**

The gene prediction and functional annotation of the genome sequence was basically performed within the Integrated Microbial Genomes – Expert Review (IMG-ER) platform [11]. The tRNAscan-SE was used to find tRNA genes [12]. Ribosomal RNA genes and ncRNA were predicted using RNAmmer [13] and Infernal [14] using the Rfam model [15], respectively. Identification of protein coding genes was performed using Prodigal [16], followed by a round of manual curation using the JGI GenePRIMP pipeline [17]. The predicted CDS were searched using the TIGR-fam, Pfam and COG databases implemented in the IMG systems.

**Genome properties**

The draft genome of *R. lacunae* KORDI 51-2T, with a total of 4.15 Mbp from 99 contigs, contains 56.22% G+C contents (Figure 3 and Table 3). A total of 3,790 genes were predicted. Of these, 283 pseudogenes. The remaining 3,457 were annotated as protein-coding genes and 50 for RNA genes (3 for rRNA, 41 for tRNA and 6 other nc RNA). The properties and the statistics of the genome are summarized in Table 3. The distribution of genes into COGs functional categories is presented in Table 4.

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**Table 2. Genome sequencing project information**

| MIGS ID | Property                     | Term                        |
|---------|------------------------------|----------------------------|
| MIGS-31 | Finishing quality            | Draft                      |
| MIGS-28 | Libraries used               | Shotgun library            |
| MIGS-29 | Sequencing platforms         | 454 GS-FLX Titanium        |
| MIGS-31.2 | Sequencing coverage         | 30x                        |
| MIGS-30 | Assemblers                   | Newbler version 2.3        |
| MIGS-32 | Gene calling method          | Prodigal, GenePRIMP       |
|         | Genbank ID                   | ASSJ000000000              |
|         | Genbank Date of Release      | October 7, 2013            |
|         | GOLD ID                      | Gi22154                    |
|         | Project relevance            | Cyanobacterial ecology     |

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Table 3. Genome statistics

| Attribute                        | Value     | % of total<sup>a</sup> |
|----------------------------------|-----------|------------------------|
| Genome size (bp)                 | 4,153,658 |                        |
| DNA Coding region (bp)           | 3,323,928 | 80.02                  |
| DNA G+C content (bp)             | 2,335,216 | 56.22                  |
| No. of contigs                   | 99        |                        |
| Total genes<sup>b</sup>          | 3,790     |                        |
| RNA genes                        | 50        | 1.32                   |
| Protein-coding genes             | 3,740     | 98.68                  |
| Genes with functional prediction | 2411      | 63.61                  |
| Genes with enzymes               | 775       | 20.45                  |
| Genes with transporter classification | 343     | 9.05                   |
| Genes assigned to COGs           | 2,228     | 58.79                  |
| Genes assigned to Pfam           | 2,511     | 66.25                  |
| Genes assigned to TIGRfam        | 976       | 25.75                  |
| Genes assigned in paralog clusters | 2427    | 64.04                  |
| Genes with signal peptides       | 137       | 3.61                   |
| Genes with transmembrane helices | 810       | 21.37                  |

<sup>a</sup> 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.

<sup>b</sup> Also includes 283 pseudogenes.

Table 4. Number of genes associated with the 25 general COG functional categories

| Code | Value | % age | Description                                         |
|------|-------|-------|-----------------------------------------------------|
| J    | 149   | 6.08  | Translation                                         |
| A    | 1     | 0.04  | RNA processing and modification                     |
| K    | 109   | 4.45  | Transcription                                       |
| L    | 127   | 5.18  | Replication, recombination and repair               |
| B    | 1     | 0.04  | Chromatin structure and dynamics                    |
| D    | 24    | 0.98  | Cell cycle control, mitosis and meiosis            |
| Y    | 0     | -     | Nuclear structure                                   |
| V    | 43    | 1.75  | Defense mechanisms                                  |
| T    | 105   | 4.28  | Signal transduction mechanisms                      |
| M    | 166   | 6.77  | Cell wall/membrane biogenesis                       |
| N    | 25    | 1.02  | Cell motility                                       |
| Z    | 1     | 0.04  | Cytoskeleton                                        |
| W    | 0     | -     | Extracellular structures                            |
| U    | 62    | 2.53  | Intracellular trafficking and secretion             |
| O    | 118   | 4.81  | Posttranslational modification, protein turnover, chaperones |
| C    | 149   | 6.08  | Energy production and conversion                    |
| G    | 126   | 5.14  | Carbohydrate transport and metabolism               |
| E    | 172   | 7.01  | Amino acid transport and metabolism                 |
| F    | 58    | 2.37  | Nucleotide transport and metabolism                 |
| H    | 157   | 6.4   | Coenzyme transport and metabolism                   |
| I    | 51    | 2.08  | Lipid transport and metabolism                      |
| P    | 158   | 6.44  | Inorganic ion transport and metabolism              |
| Q    | 75    | 3.06  | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 331   | 13.5  | General function prediction only                    |
| S    | 244   | 9.95  | Function unknown                                    |
|     | 1562  | 41.21 | Not in COGs                                         |
**Insights from the genome sequence**

A genome analysis of *R. lacunae* KORDI 51-2T, revealed that it contains a gene cluster participating in organic phosphonate utilization. Likewise with a marine nitrogen-fixing cyanobacterium, *Trichodesmium erythraeum* IMS101 [18], the strain KORDI 51-2T has orthologs to *phnC-E* (transporters) and *phnG-M* (C-P lyase complex) (Figure 4A). Additionally, an ortholog to *phnF* (transcriptional regulator) is found in strain KORDI 51-2T, but not in *T. erythraeum* IMS101. Phylogenetic analysis of PhnJ proteins found in various bacterial strains, showed that PhnJ proteins of cyanobacteria form polyphyletic lineages (Figure 4B), suggesting that the *phn* gene cluster of cyanobacteria might be acquired by horizontal gene transfer. As KORDI 51-2T can grow in media supplemented with variety of organic phosphonate substrates (2-aminoethylphosphonate, methylphosphonate, phosphonoacetic acid and phosphonformic acid) as a sole P-source (data not shown), the strain must be able to cleave C-P bonds of organic phosphonate by C-P lyase pathways and utilize them as a P-source.

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**Figure 3.** Graphical circular map of the genome. From outside to the center: color by COG categories and RNAs on forward strand, genes on forward strand, genes on reverse strand, color by COG categories and RNAs on reverse strand, GC content, GC skew.
Figure 4. DNA topology of the *phn* cluster (A) and phylogenetic analysis of the PhnJ protein (B). A, Genes encoding phosphonate transport (gray), regulation (light gray), and the C-P lyase subunits (dark gray) are shown. Additional two sets of transporters were not shown. B, Phylogenetic relationship of the PhnJ protein from a variety of bacteria determined by maximum-likelihood analysis. Bootstrap values >70 are shown at the nodes. The scale bar represents amino-acid substitution per site.

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References

1. Choi DH, Noh JH, Lee CM, Rho S. *Rubidibacter lacunae* gen. nov., sp. nov., a unicellular, phycocerythrin-containing cyanobacterium isolated from seawater of Chuuk lagoon, Micronesia. *Int J Syst Evol Microbiol* 2008; 58:2807-2811. [PubMed](http://dx.doi.org/10.1099/ijs.0.65798-0)

2. Garcia-Pichel F, Nübel U, Muyzer G. The phylogeny of unicellular, extremely halotolerant cyanobacteria. *Arch Microbiol* 1998; 169:469-482. [PubMed](http://dx.doi.org/10.1007/s002030050599)

3. Allen MA, Goh F, Burns BP, Neilan BA. Bacterial, archaeal and eukaryotic diversity of smooth and pustular microbial mat communities in the hypersaline lagoon of Shark Bay. *Geobiology* 2009; 7:82-96. [PubMed](http://dx.doi.org/10.1111/j.1472-4669.2008.00187.x)
Rubidibacter lacunae strain KORDI 51-2T

4. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV, et al. The minimum information about a genome sequence (MIGS) specification. *Nat Biotechnol* 2008; 26:541-547. PubMed http://dx.doi.org/10.1038/nbt1360

5. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc Natl Acad Sci USA* 1990; 87:4576-4579. PubMed http://dx.doi.org/10.1073/pnas.87.12.4576

6. Castenholz RW. 2001. Oxygenic photosynthetic bacteria. In: Garrity GM, Boone DR, Castenholz RW (eds) *Bergey’s Manual of Systematic Bacteriology* 2nd ed. Vol 1, Springer-Verlag, New York, pp. 473-600.

7. McNeill J, Barrie FR, Burdet HM, Demoulin V, Hawksworth DL, Marhold K, Nicolson DH, Prado J, Silva PC, Skog JE, et al. International Code of Botanical Nomenclature, A.R.G. Ganter, Königstein, 2006, p. 1.

8. Woese CR, Stackebrandt E, Macke TJ, Fox GE. A phylogenetic definition of the major eubacterial taxa. *Syst Appl Microbial* 1985; 6:143-151. PubMed http://dx.doi.org/10.1016/S0723-2020(85)80047-3

9. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; 25:25-29. PubMed http://dx.doi.org/10.1038/75556

10. Liolios K, Chen IM, Mavromatis K, Tavernarakis N, Hugenholtz P, Markowitz VM, Kyprides NC. The Genomes On Line Database (GOLD) in 2009: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2010; 38:D346-D354. PubMed http://dx.doi.org/10.1093/nar/gkp848

11. Markowitz VM, Mavromatis K, Nvanova NN, Chen IM, Chu K, Kyprides NC. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* 2009; 25:2271-2278. PubMed http://dx.doi.org/10.1093/bioinformatics/btp393

12. Lowe TM, Eddy SR. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res* 1997; 25:955-964. PubMed

13. Lagesen K, Hallin P, Rødland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmer: consistent and rapid annotation of ribosomal RNA genes. *Nucleic Acids Res* 2007; 35:3100-3108. PubMed http://dx.doi.org/10.1093/nar/gkm160

14. Nawrocki EP, Kolbe DL, Eddy SR. Infernal 1.0: inference of RNA alignments. *Bioinformatics* 2009; 25:1335-1337. PubMed http://dx.doi.org/10.1093/bioinformatics/btp157

15. Griffiths-Jones S, Moxon S, Marshall M, Khanna A, Eddy SR, Bateman A. Rfam: annotating non-coding RNAs in complete genomes. *Nucleic Acids Res* 2005; 33:D121-D124. PubMed http://dx.doi.org/10.1093/nar/gki081

16. Hyatt D, Chen GL, LoCascio P, Land M, Larimer F, Hauser L. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 2010; 11:119. PubMed http://dx.doi.org/10.1186/1471-2105-11-119

17. Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyprides NC. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. *Nat Methods* 2010; 7:455-457. PubMed http://dx.doi.org/10.1038/nmeth.1457

18. Dyhrman ST, Chappell PD, Haley ST, Moffett JW, Orchard ED, Waterbury JB, Webb EA. Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. *Nature* 2006; 439:68-71. PubMed http://dx.doi.org/10.1038/nature04203