Complete genome sequence of *Halogeometricum borinquense* type strain (PR3<sup>T</sup>)

Stephanie Malfatti<sup>1,2</sup>, Brian J. Tindall<sup>3</sup>, Susanne Schneider<sup>3</sup>, Regine Fähnrich<sup>1</sup>, Alla Lapidus<sup>1</sup>, Kurt LaButti<sup>1</sup>, Alex Copeland<sup>1</sup>, Tijana Glavina Del Rio<sup>1</sup>, Matt Nolan<sup>1</sup>, Feng Chen<sup>1</sup>, Susan Lucas<sup>1</sup>, Hope Tice<sup>1</sup>, Jan-Fang Cheng<sup>1</sup>, David Bruce<sup>1,4</sup>, Lynne Goodwin<sup>1,4</sup>, Sam Pitluck<sup>1</sup>, Iain Anderson<sup>1</sup>, Amrita Pati<sup>1</sup>, Natalia Ivanova<sup>1</sup>, Konstantinos Mavromatis<sup>1</sup>, Amy Chen<sup>3</sup>, Markus Paliyapan<sup>2</sup>, Patrik D’haeseleer<sup>1,2</sup>, Markus Göker<sup>3</sup>, Jim Bristow<sup>1</sup>, Jonathan A. Eisen<sup>1,6</sup>, Victor Markowitz<sup>5</sup>, Philip Hugenholtz<sup>1</sup>, Nikos C. Kyrpides<sup>1</sup>, Hans-Peter Klenk<sup>3</sup>, and Patrick Chain<sup>1,2</sup>*

1 DOE Joint Genome Institute, Walnut Creek, California, USA  
2 Lawrence Livermore National Laboratory, Livermore, California, USA  
3 DSMZ - German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany  
4 Los Alamos National Laboratory, Bioscience Division, Los Alamos, New Mexico, USA  
5 Biological Data Management and Technology Center, Lawrence Berkeley National Laboratory, Berkeley, California, USA  
6 University of California Davis Genome Center, Davis, California, USA  

*Corresponding author: Patrick Chain

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*Halogeometricum borinquense* Montalvo-Rodríguez et al. 1998 is the type species of the genus, and is of phylogenetic interest because of its distinct location between the halobacterial genera *Haloquadratum* and *Halosarcina*. *H. borinquense* requires extremely high salt (NaCl) concentrations for growth. It can not only grow aerobically but also anaerobically using nitrate as electron acceptor. The strain described in this report is a free-living, motile, pleomorphic, euryarchaeon, which was originally isolated from the solar salterns of Cabo Rojo, Puerto Rico. Here we describe the features of this organism, together with the complete genome sequence, and annotation. This is the first complete genome sequence of the halobacterial genus *Halogeometricum*, and this 3,944,467 bp long six replicon genome with its 3937 protein-coding and 57 RNA genes is part of the Genomic Encyclopedia of Bacteria and Archaea project.

Introduction

Strain PR3<sup>T</sup> (= DSM 11551 = ATCC 700274 = JCM 10706) is the type strain of *Halogeometricum borinquense*, representing the sole species of the genus *Halogeometricum* [1]. Strain PR3<sup>T</sup> was first described by Montalvo-Rodriguez et al. in 1998 [1] as Gram-stain negative and motile. The organism is of interest because of its position in the tree of life, where it is located between members of the *Haloferax/Halorubrum* cluster within the large euryarchaeal family *Halobacteraceae* (Figure 1). Here we present a summary classification and a set of features for *H. geometricum* PR3<sup>T</sup> together with the description of the complete genomic sequencing and annotation.

Classification and features

In addition to the solar salterns of Cabo Rojo, Puerto Rico, where the type strain PR3<sup>T</sup> and two accompanying strains (PR7 and PR9) were initially isolated [1], the occurrence of strains or phylotypes closely related or belonging to *H. borinquense* have so far only been reported from high salt environments such as an Australian crystallizer pond [6], Maras Salterns in the Peruvian Andes [7], a salt field at Nie, Ishikawa Prefecture, Japan [8], the salterns of Tamilnadu, India (Kannan et al. unpublished), Exportadora del Sal, Guerrero Negro, Mexico (FJ609942), a Taiwanese saltern soil (FJ348412), and a low-salt, sulfide- and sulfur-rich spring in southwestern Oklahoma, USA [9].
H. geometricum PR3\(^T\) cells are highly pleomorphic (short and long rods, squares, triangles and ovals) and motile by peritrichous flagella (Table 1 and Figure 2). Cells lyse in distilled water. Gas vesicles are present and are responsible for modifying the color of colonies or cell suspensions from red to pink. H. geometricum PR3\(^T\) is aerobic, but also capable of anaerobic growth with nitrate. No anaerobic growth on arginine (arginine dihydrolase is not present). At least 8% NaCl (w/v) is required for growth, reflecting the primary characteristic requirement for high salt concentrations of the Halobacteriaceae [18]. The optimal NaCl concentration range is 20-25% NaCl (w/v) at 40°C (optimal growth temperature). Nitrate is reduced to nitrite with the production of gas [1]. Spores or other resting stages have not been reported [1]. H. geometricum PR3\(^T\) is capable of degrading gelatin, but starch is not hydrolysed. A number of sugars and polyols are used as carbon sources, and acid is produced from some sugars [1].

Figure 1 shows the phylogenetic neighborhood of H. borinquense strain PR3\(^T\) in a 16S rRNA based tree. Analysis of the two 16S rRNA gene sequences in the genome of strain PR3\(^T\) indicated that the two genes differ by five nucleotides (nts) from each other, and by 3-5 nts from the previously published 16S rRNA sequence generated from DSM 11551 (AF002984). The slight differences between the genome data and the reported 16S rRNA gene sequence are most likely the result of sequencing errors in the previously reported sequence data.

**Chemotaxonomy**

The quinone composition of H. borinquense strain PR3\(^T\) has not been recorded, but based on reports from other members of the family Halobacteriaceae menaquinones with eight isoprenoid units are likely to be present. Typically both MK-8 and MK-8 (VII-H\(_2\)) are predicted. The lipids are based on isoprenoid diether lipids, but the exact nature of the isoprenoid side chains remains to be investigated. The major phospholipids are the diether, isoprenoid analogs of phosphatidylglycerol and methyl-phosphatidylglycerophosphate (typical of all members of the family Halobacteriaceae), the diether analog of phosphatidylglycerol sulfate is absent [1]. A single glycolipid has been reported with an R\(_f\) value similar to that of the triglycosyl diether from Haloarcula marismortui, but its structure has not been determined [1]. The pigments responsible for the red color of the cells have not been determined, but it may be predicted that they are carotenoids, probably bacterioruberins. Outer cell layers are probably proteinaceous. The presence of peptidoglycan has not been investigated, but is generally absent from members of this family Halobacteriaceae.

Figure 1. Phylogenetic tree of H. borinquense PR3\(^T\) with a selection of type strains of the family Halobacteriaceae, inferred from 1,433 aligned 16S rRNA characters [2] under the maximum likelihood criterion [3,4]. The tree was rooted with Natronomonas pharaonis, the deepest branching member of the family Halobacteriaceae. The branches are scaled in terms of the expected number of substitutions per site. Numbers above branches are support values from 1,000 bootstrap replicates. Strains with a genome sequencing project registered in GOLD [5] are printed in blue; published genomes in bold.

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**Table 1. Classification and general features of *H. borinquense* PR3^T according to the MIGS recommendations [10]**

| MIGS ID | Property                  | Term                                                                 | Evidence code |
|---------|---------------------------|----------------------------------------------------------------------|---------------|
|         | Domain                     | Archaea                                                             | TAS [11]      |
|         | Phylum                     | Euryarchaeota                                                        | TAS [12]      |
|         | Class                      | Halobacteria                                                         | TAS [13]      |
|         | Order                      | Halobacteriales                                                      | TAS [14]      |
|         | Family                     | Halobacteriaceae                                                    | TAS [15]      |
|         | Genus                      | Halogeometricum                                                     | TAS [1]       |
|         | Species                    | *Halogeometricum borinquense*                                        | TAS [1]       |
|         | Type strain                | PR3                                                                 | TAS [1]       |
|         | Gram stain                 | negative                                                            | TAS [1]       |
|         | Cell shape                 | highly pleomorphic                                                  | TAS [1]       |
|         | Motility                   | motile                                                              | TAS [1]       |
|         | Sporulation                | non-sporulating                                                     | NAS           |
|         | Temperature range          | mesophile, between 22°C and 50°C                                     | TAS [1]       |
|         | Optimum temperature        | 40°C                                                                | TAS [1]       |
|         | Salinity                   | halophile, at least 8% (w/v) NaCl                                    | TAS [1]       |
| MIGS-22 | Oxygen requirement         | primarily aerobic; facultatively anaerobic growth via nitrate reduction | TAS [1]       |
|         | Carbon source              | glucose, mannose, fructose, xylose, maltose, trehalose, cellobiose, raffinose, glycerol | TAS [1]       |
|         | Energy source              | carbohydrates                                                       | TAS [1]       |
|         | Habitat                    | aquatic                                                              | TAS [1]       |
| MIGS-6  | Biotic relationship        | free living                                                         | NAS           |
| MIGS-15 | Pathogenicity              | none                                                                | NAS           |
| MIGS-14 | Biosafety level            | 1                                                                   | TAS [16]      |
|         | Isolation                  | solar salters of Cabo Rojo, Puerto Rico                             | TAS [1]       |
| MIGS-4  | Geographic location        | Cabo Rojo, Puerto Rico                                              | TAS [1]       |
| MIGS-5  | Sample collection time     | 1994                                                               | TAS [1]       |
| MIGS-4.1| Latitude / Longitude       | 18,088 / -67,147                                                    | TAS [1]       |
| MIGS-4.2| Altitude                   | sea level                                                           | NAS           |
| MIGS-4.3| Depth                      | not reported                                                        |               |
| MIGS-4.4|                           |                                                                     |               |

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); 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 [17]. If the evidence code is IDA then the property was directly observed for a living isolate by one of the authors or an expert mentioned in the acknowledgements.
Genome sequencing and annotation

Genome project history
This organism was selected for sequencing on the basis of each phylogenetic position, and is part of the Genomic Encyclopedia of Bacteria and Archaea project. The genome project is deposited in the Genome OnLine Database [5]. The complete genome sequence has not yet been released from GenBank. Sequencing, finishing and annotation were performed by the DOE Joint Genome Institute (JGI). A summary of the project information is shown in Table 2.

Table 2. Genome sequencing project information

| MIGS ID | Property                  | Term                                                                 |
|---------|---------------------------|----------------------------------------------------------------------|
| MIGS-31 | Finishing quality         | Finished two genomic libraries: 8kb pMCL200                           |
|         |                            | and fosmid pcc1Fos Sanger libraries. one 454 pyrosequence standard   |
|         |                            | library.                                                             |
| MIGS-28 | Libraries used            | Two genomic libraries: 8kb pMCL200                                   |
|         |                            | and fosmid pcc1Fos Sanger libraries. one 454 pyrosequence standard   |
|         |                            | library.                                                             |
| MIGS-29 | Sequencing platforms      | ABI3730, 454 GS FLX                                                   |
| MIGS-31.2 | Sequencing coverage    | 9.7× Sanger; 21.8× pyrosequencing                                    |
| MIGS-30 | Assemblers                | Newbler, PGA                                                         |
| MIGS-32 | Gene calling method       | GeneMark 4.6b, tRNAscan-SE-1.23, internal 0.81                      |
| INSDC / Genbank ID |                      | CP001688                                                            |
| Genbank Date of Release |                | September 10, 2009                                                   |
| GOLD ID  |                          | Gc01108                                                             |
| NCBI project ID |                       | 20743                                                               |
| Database: IMG-GEBA |                | 2501416934                                                          |
| MIGS-13  | Source material identifier | DSM 11551                                                          |
| Project relevance |              | Tree of Life, GBEA                                                  |

Growth conditions and DNA isolation
H. borinquense PR3⁷, DSM 11551, was grown in DSMZ medium 372 (Halobacteria Medium) at 35°C [19]. DNA was isolated from 1-1.5 g of cell paste using a Qiagen Genomic 500 DNA Kit (Qiagen, Hilden, Germany) with a modified protocol for cell lysis, LALMP procedure according to Wu et al. [20].

Genome sequencing and assembly
The genome was sequenced using a combination of Sanger and 454 sequencing platforms. All general aspects of library construction and sequencing performed at the JGI can be found at http://www.jgi.doe.gov/. 454 Pyrosequencing reads were assembled using the Newbler assembler version v 2.0.0 (Roche). Large Newbler contigs were broken into 4,435 overlapping fragments of 1,000 bp and entered into assembly as pseudo-reads. The sequences were assigned quality scores based on Newbler consensus q-scores with modifications to account for overlap redundancy and adjust inflated q-scores. A hybrid 454/Sanger assembly was made using the PGA assembler. Possible mis-assemblies were corrected and gaps between contigs were closed by custom primer walks from sub-clones or PCR products. A total of 2,826 Sanger finishing reads were produced. The error rate of the completed genome sequence is less than 1 in 100,000. Together all sequence types provided 31.5× coverage of the genome.

Genome annotation
Genes were identified using GeneMark [21] as part of the genome annotation pipeline in the Integrated Microbial Genomes Expert Review (IMG-ER) system [22], followed by a round of manual curation using the JGI GenePRIMP pipeline [23]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. The tRNAscanSE tool [24] was used to find tRNA genes, whereas ribosomal RNAs were found by using the tool RNAmmer [25]. Other non-coding RNAs were identified by searching the ge-

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Halogeometricum borinquense type strain (PR3)

nome for the Rfam profiles using INFERNAL (v0.81) [26]. Additional gene prediction analysis and manual functional annotation was performed within the Integrated Microbial Genomes (IMG) platform [27].

**Metabolic network analysis**
The metabolic Pathway/Genome Database (PGDB) was computationally generated using Pathway Tools software version 12.5 [28] and MetaCyc version 12.5 [29], based on annotated EC numbers and a customized enzyme name mapping file. It has undergone no subsequent manual curation and may contain errors, similar to a Tier 3 BioCyc PGDB [30].

**Genome properties**
The genome is 3,944,467 bp long and comprises one main circular chromosome with a 60% GC content and five plasmids. Of the 3,994 genes predicted, 3,937 were protein coding genes, and 57 RNAs. Thirty seven pseudogenes were also identified. A total of 62% of the genes were assigned a putative function while the remaining ones are annotated as hypothetical proteins. The properties and the statistics of the genome are summarized in Table 3. The distribution of genes into COGs functional categories is presented in Figure 3 and Table 4. A cellular overview diagram is presented in Figure 4, followed by a summary of metabolic network statistics shown in Table 5.

![Figure 3. Graphical circular map of the genome. From outside to the center: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNAs green, rRNAs red, other RNAs black), GC content, GC skew.]
### Table 3. Genome Statistics

| Attribute                     | Value    | % of Total |
|-------------------------------|----------|------------|
| Genome size (bp)              | 3,944,467| 100.00%    |
| DNA Coding region (bp)        | 3,441,571| 87.25%     |
| DNA G+C content (bp)          | 2,364,339| 59.94%     |
| Number of replicons           | 1        |            |
| Extrachromosomal elements     | 5        |            |
| Total genes                   | 3994     | 100.00%    |
| RNA genes                     | 57       | 1.90%      |
| rRNA operons                  | 2        |            |
| Protein-coding genes          | 3937     | 98.57%     |
| Pseudogenes                   | 37       | 0.93%      |
| Genes with function prediction| 2486     | 62.24%     |
| Genes in paralog clusters     | 741      | 18.55%     |
| Genes assigned to COGs        | 2449     | 61.32%     |
| Genes assigned Pfam domains   | 2385     | 59.71%     |
| Genes with signal peptides    | 533      | 13.35%     |
| Genes with transmembrane helices| 971    | 24.31%     |
| CRISPR repeats                | 1        |            |

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

| Code | Value | % age | Description                                                                 |
|------|-------|-------|-----------------------------------------------------------------------------|
| J    | 162   | 4.1   | Translation, ribosomal structure and biogenesis                            |
| A    | 1     | 0.0   | RNA processing and modification                                             |
| K    | 140   | 3.6   | Transcription                                                               |
| L    | 138   | 3.5   | Replication, recombination and repair                                       |
| B    | 3     | 0.0   | Chromatin structure and dynamics                                            |
| D    | 0     | 0.1   | Cell cycle control, mitosis and meiosis                                     |
| Y    | 0     | 0.0   | Nuclear structure                                                           |
| V    | 46    | 1.2   | Defense mechanisms                                                          |
| T    | 113   | 2.8   | Signal transduction mechanisms                                              |
| M    | 87    | 2.2   | Cell wall/membrane biogenesis                                               |
| N    | 38    | 0.1   | Cell motility                                                               |
| Z    | 0     | 0.0   | Cytoskeleton                                                                |
| W    | 0     | 0.0   | Extracellular structures                                                    |
| U    | 27    | 0.7   | Intracellular trafficking and secretion                                     |
| O    | 123   | 3.1   | Posttranslational modification, protein turnover, chaperones                |
| C    | 174   | 4.4   | Energy production and conversion                                            |
| G    | 124   | 3.1   | Carbohydrate transport and metabolism                                       |
| E    | 271   | 6.9   | Amino acid transport and metabolism                                         |
| F    | 77    | 1.9   | Nucleotide transport and metabolism                                         |
| H    | 140   | 3.5   | Coenzyme transport and metabolism                                          |
| I    | 98    | 2.5   | Lipid transport and metabolism                                              |
| P    | 178   | 4.5   | Inorganic ion transport and metabolism                                       |
| Q    | 60    | 1.5   | Secondary metabolites biosynthesis, transport and catabolism                |
| R    | 433   | 11.0  | General function prediction only                                            |
| S    | 227   | 5.8   | Function unknown                                                            |
|     | 1488  | 37.8  | Not in COGs                                                                 |

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Figure 4. Schematic cellular overview diagram of all pathways of H. borinquense strain PR3T. Nodes represent metabolites, with shape indicating class of metabolite. Lines represent reactions.

Table 5. Metabolic Network Statistics

| Attribute            | Value |
|----------------------|-------|
| Total genes          | 3801  |
| Enzymes              | 578   |
| Enzymatic reactions  | 687   |
| Metabolic pathways   | 125   |
| Metabolites          | 578   |

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References

1. Montalvo-Rodríguez R, Vreeland RH, Oren A, Kesse M, Betancourt C, López-Garriga J. Halogeometricum borinquense gen. nov., sp. nov., a novel halophilic archaeon from Puerto Rico. Int J Syst Bacteriol 1998; 48:1305-1312. PubMed

2. Lee C, Grasso C, Sharlow MF. Multiple sequence alignment using partial order graphs. Bioinformatics 2002; 18:452-464. PubMed

3. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 1981; 17:368-376. PubMed

4. Stamatakis A, Hoover P, Rougemont J. A rapid bootstrap algorithm for the RAxML web-servers. Syst Biol 2008; 57:758-771. PubMed

5. Liolios K, Mavromatis K, Tavernarakis N, Kyrpides NC. The Genomes OnLine Database (GOLD) in 2007: status of genomic and metagenomic projects and their associated metadata. Nucleic Acids Res 2008; 36:D475-D479. PubMed doi:10.1093/nar/gkm884

6. Maturrano L, Santos F, Rosselló-Mora R, Antón J. Microbial diversity in Maras Salterns, a hypersaline environment in the Peruvian Andes. Appl Environ Microbiol 2006; 72:3887-3895. PubMed doi:10.1128/AEM.02214-05

7. Fukushima T, Usami R, Kamekura M. A traditional Japanese-style salt field is a niche for haloarchaeal strains that can survive in 0.5% salt solution. Saline Systems 2007; 72:3887-3895.
low-salt, sulphide- and sulphur-rich spring. Appl Environ Microbiol 2004; 70:2230-2239. PubMed doi:10.1128/AEM.70.4.2230-2239.2004

10. Field D, Garrity G, Gray T, Morrison N, Selengut J, Sterk P, Tatusova T, Thomson N, Allen MJ, Angiuoli SV et al. Towards a richer description of our complete collection of genomes and metagenomes: the “Minimum Information about a Genome Sequence” (MIGS) specification. Nat Biotechnol 2008; 26:541-547. PubMed doi:10.1038/nbt1360

11. 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 doi:10.1073/pnas.87.12.4576

12. Garrity GM, Lilburn TG, Cole JR, Harrison SH, Ezéby J, Tindall BJ. Taxonomic Outline of the Bacteria and Archaea. Release 7.7 March 6, 2007. Michigan State University Board of Trustees. DOI: 10.1601/TOBA7.7. http://www.taxonomicoutline.org/index.php/toba

13. Grant WD, Kamekura M, McGinity TJ, Ventosa A. Class III. Halobacteria class. nov. In: DR Boone, RW Castenholz, GM Garrity (eds): Bergey’s Manual of Systematic Bacteriology, 2nd edition, Vol. 1, The Archaea and the deeply branching and phototrophic Bacteria, Springer-Verlag, New York, 2001.p 294.

14. Grant WD, Larsen H. Group III. Extremely halophilic archaeobacteria. Order Halobacteriales ord. nov. In: JT Staley, MP Bryant, N. Pfennig, JG Holt (eds), Bergey’s Manual of Systematic Bacteriology, 1st edition, Vol. 3, The Williams & Wilkins Co., Baltimore, 1989, pp. 2216-2218.

15. Gibbons NE. Family V. Halobacteriaceae fam. nov. In: RE Buchanan, NE Gibbons (eds): Bergey’s Manual of Determinative Bacteriology, 8th edition, The Williams & Wilkins Co, Baltimore, 1974, p. 269.

16. Anonymous. Biological Agents: Technical rules for biological agents www.baua.de TRBA 466.

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

18. Norton C. Rediscovering the ecology of halobacteria. ASM News 1992; 58:363-367.

19. List of media used at DSMZ for cell growth: http://www.dsmz.de/microorganisms/media_list.php

20. Wu M, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova N, Kunin V, Goodwin I, Wu M, Tindall BJ, et al. A phylogeny-driven genomic encyclopedia of Bacteria and Archaea. Nature 2009 (In press)

21. Besemer J, Lomsadze A, Borodovsky M. GeneMarkS: a self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res 2001; 29:2607-2618. PubMed PubMed doi:10.1093/nar/29.12.2607

22. Markowitz VM, Mavromatis K, Ivanova NN, Chen IM, Chu K, Kyrpides NC. Expert Review of Functional Annotations for Microbial Genomes. Bioinformatics 2009 25:2271-2278.

23. Patti A, Ivanova N, Mikhailova N, Ovchinkova G, Hooper SD, Lykidis A, Kyrpides NC. GenePRIMP: A Gene Prediction Improvement Pipeline for microbial genomes. (Submitted) 2009.

24. 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 doi:10.1093/nar/25.5.955

25. Lagesen K, Hallin P, Redland EA, Staerfeldt HH, Rognes T, Ussery DW. RNAmmer: consistent and rapid annotation of ribosomal RNA genes. Nucleic Acids Res 2007; 35:3100-3108. PubMed doi:10.1093/nar/gkm160

26. 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 doi:10.1093/nar/gki081

27. Markowitz VM, Szeto E, Palaniappan K, Grechkin Y, Chu K, Chen IMA, Dubchak I, Anderson I, Lykidis A, Mavromatis K, et al. The Integrated Microbial Genomes (IMG) system in 2007: data content and analysis tool extensions. Nucleic Acids Res 2008; 36:D528-D533. PubMed doi:10.1093/nar/gkm846

28. Karp PD, Paley SM, Romero P. The Pathway Tools Software. Bioinformatics 2002; 18:S225-S232. PubMed

29. Caspi R, Karp P, Foerster H, Fulcher CA, Kaipa P, Krümenacker M, Latendresse M, Paley SM, Rhee SY, Shearer A, et al. The MetaCyc Database of metabolic pathways and enzymes and the BioCyc collection of pathway/Genome Databases. Nucleic
*Halogeometricum borinquense* type strain (PR3)

*Acids Res* 2008; 36:D623-D631. PubMed doi:10.1093/nar/gkm900

30. Karp PD, Ouzounis CA, Moore-Kochlacs C, Goldovsky L, Kaipa P, Ahren D, Tsoka S, Darzentas N, Kunin V, Lopez-Bigas N. Expansion of the BioCyc collection of pathway/genome databases to 160 genomes. *Nucleic Acids Res* 2005; 33:6083-6089. PubMed doi:10.1093/nar/gki892