Complete genome sequence of the facultatively anaerobic, appended bacterium Muricauda ruestringensis type strain (B1T)

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Abstract
Muricauda ruestringensis Bruns et al. 2001 is the type species of the genus Muricauda, which belongs to the family Flavobacteriaceae in the class phylum “Bacteroidetes”. The species is of interest because of its isolates position in the already genome-sequenced part of the tree of life in a genomically so far uncharted genus. This is the first completed genome sequence of a member of the genus Muricauda. The genome, which consists of a circular chromosome of 3,842,422 bp length with a total of 3,478 protein-coding and 47 RNA genes, is a part of the Genomic Encyclopedia of Bacteria and Archaea project.

Introduction
Strain B1T (= DSM 13258 = LMG 19739 = KCTC 12928) is the type strain of the species Muricauda ruestringensis, which is the type species of the currently six species containing genus Muricauda [1,20]. The genus name was derived from the Latin words muris, of the mouse, and cauda, the tail; Muricauda, tail of the mouse, referring to the cellular appendages observed on some cells [1]. The species epithet is derived from the Neo-Latin word ruestringensis, pertaining to to the former village of Rüstringen, which was destroyed by a tidal wave in 1362 [1]. Stain B1T was isolated from a seawater sediment suspension from intertidal sediment at the German North Sea coast, containing hexadecane as sole carbon source during the initial cultivation, but later turned out to be not able to degrade hexadecane
Other isolates belonging the species are not known, nor was strain B1\textsuperscript{T} used for scientific work other than the description of the species \textit{M. ruestringensis}. Here we present a summary classification and a set of features for \textit{M. ruestringensis} strain B1\textsuperscript{T}, together with the description of the complete genomic sequencing and annotation.

**Classification and features**

A representative genomic 16S rRNA sequence of \textit{M. ruestringensis} B1\textsuperscript{T} was compared using NCBI BLAST [7,8] under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent release of the Greengenes database [9] and the relative frequencies of taxa and keywords (reduced to their stem [10]) were determined, weighted by BLAST scores. The most frequently occurring genera were \textit{Muricauda} (24.7\%), \textit{Maribacter} (24.0\%), \textit{Cytophaga} (12.3\%), \textit{Zobellia} (9.6\%) and \textit{Flavobacterium} (7.1\%) (118 hits in total). Regarding the two hits to sequences from members of the species, the average identity within HSPs was 99.7\%, whereas the average coverage by HSPs was 93.8\%. Regarding the six hits to sequences from other members of the genus, the average identity within HSPs was 97.9\%, whereas the average coverage by HSPs was 97.9\%. Among all other species, the one yielding the highest score was \textit{Muricauda aquimarina} (EU440979), which corresponded to an identity of 98.7\% and an HSP coverage of 98.4\%. (Note that the Greengenes database uses the INSDC (= EMBL/NCBI/DDBJ) annotation, which is not an authoritative source for nomenclature or classification.) The highest-scoring environmental sequence was HQ326265 ('Microbial structure biofilm on SWRO membranes clone SBS-FW-047'), which showed an identity of 98.5\% and an HSP coverage of 98.0\%. The most frequently occurring keywords within the labels of all environmental samples which yielded hits were 'microbi' (4.7\%), 'sediment' (4.1\%), 'sea' (2.9\%), 'marin' (2.4\%) and 'biofilm' (2.4\%) (132 hits in total). Environmental samples which yielded hits of a higher score than the highest scoring species were not found.

Figure 1 shows the phylogenetic neighborhood of \textit{M. ruestringensis} in a 16S rRNA based tree. The sequences of the two identical 16S rRNA gene copies in the genome differ by one nucleotide from the previously published 16S rRNA sequence (AF218782).
Figure 1. Phylogenetic tree highlighting the position of *M. ruestringensis* relative to the type strains of the other species within the genus *Muricauda*. The tree was inferred from 1,481 aligned characters [18,19] of the 16S rRNA gene sequence under the maximum likelihood (ML) criterion [13]. *Flavobacterium aquatile* was included in the dataset for use as outgroup taxa. The branches are scaled in terms of the expected number of substitutions per site. Numbers adjacent to the branches are support values from 850 ML bootstrap replicates [21] (left) and from 1,000 Maximum-Parsimony bootstrap replicates [14] (right) if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [22] are labeled with one asterisk, those also listed as 'Complete and Published' with two asterisks.

Cells of strain B1<sup>T</sup> are rod-shaped with rounded ends, 0.3 - 0.6 µm wide and 1.1 - 2.7 µm long (Figure 2) [1]. Cells of older cultures are characterized by mostly polar-located appendages with vesicle-like structures (blebs) at the end (Figure 2), which were discussed in detail by Bruns et al. in [1] and probably serve to contact cells to each other or for colonization of a substratum [1]. The non-motile cells (see missing genes in category motility in Table 4) stain Gram-negative and grow facultatively anaerobic in seawater. The temperature range for growth is between 8°C and 40°C, with an optimum between 20 and 30°C [1]. The pH range for growth is 6.0-8.0, with an optimum at pH 6.5-7.5 [1]. Physiology and metabolism are discussed in detail in [1], with the surprising discovery that although strain B1<sup>T</sup> was isolated from a continuous-flow culture containing hexadecane as sole carbon source the strain was unable to degrade hexadecane (and other high-molecular-mass carbohydrates); neither could it use acetate or pyruvate, but a wide spectrum of amino acids as carbon and energy sources [1].

![Figure 2. Scanning electron micrograph of *M. ruestringensis* B1<sup>T</sup>](image)

**Chemotaxonomy**

The spectrum of whole-cell fatty acids represents the only chemotaxonomical data so far published for strain B1<sup>T</sup>. The spectrum of acids was clearly dominated by branched-chain acids (72%): 3-OH-iso-C<sub>17:0</sub> (28.7%), iso-C<sub>15:1</sub> (16.3%), iso-C<sub>15:0</sub> (15.5%), 3-OH-iso-C<sub>15:0</sub> (4.9%), 3-OH-iso-C<sub>16:0</sub> (2.9%), 2-OH-iso-C<sub>17:0</sub> (2.8%), 2-OH-iso-C<sub>15:0</sub> (2.5%), C<sub>16:1-7c</sub> (2.5%), anteiso-C<sub>15:0</sub> (2.4%), other acids below 2% [1].
Table 1. Classification and general features of *M. ruestringensis* B1\(^+\) in accordance with the MIGS recommendations [27].

| MIGS ID | Property                  | Term                                      | Evidence code |
|---------|---------------------------|-------------------------------------------|---------------|
|         | Current classification    | Domain *Bacteria*                          | TAS [28]      |
|         |                            | Phylum “*Bacteroidetes*”                   | TAS [29]      |
|         |                            | Class *Flavobacteria*                     | TAS [4-6]     |
|         |                            | Order “*Flavobacterales*”                 | TAS [11,12,15]|
|         |                            | Family *Flavobacteraceae*                | TAS [23-26]   |
|         |                            | Genus *Muricauda*                         | TAS [1-3]     |
|         |                            | Species *Muricauda ruestringensis*       | TAS [1]       |
|         |                            | Type strain B1                            | TAS [1]       |
|         | Gram stain                | negative                                  | TAS [1]       |
|         | Cell shape                | rod-shaped                                | TAS [1]       |
|         | Motility                  | non-motile                                | TAS [1]       |
|         | Sporulation               | not reported                              | TAS [1]       |
|         | Temperature range         | mesophile, 20°C–30°C                      | TAS [1]       |
|         | Optimum temperature       | 30°C                                      | TAS [1]       |
|         | Salinity                  | slightly halophilic, optimum 3% NaCl (w/v)| TAS [1]       |
| MIGS-22 | Oxygen requirement        | facultatively anaerobic                   | TAS [1]       |
|         | Carbon source             | various sugars and amino acids            | TAS [1]       |
|         | Energy metabolism         | chemoheterotroph                          | TAS [1]       |
| MIGS-6  | Habitat                   | marine                                    | TAS [1]       |
| MIGS-15 | Biotic relationship       | free-living                               | TAS [1]       |
| MIGS-14 | Pathogenicity             | none                                      | NAS           |
|         | Biosafety level           | 1                                         | TAS [30]      |
|         | Isolation                 | seawater sediment suspension              | TAS [1]       |
| MIGS-4  | Geographic location       | Jadebusen Bay, coast of North Sea, Germany| TAS [1]       |
| MIGS-5  | Sample collection time    | 2001 or earlier                           | NAS           |
| MIGS-4.1| Latitude                  | 53.45                                     | NAS           |
| MIGS-4.2| Longitude                 | 8.20                                      | NAS           |
| MIGS-4.3| Depth                    | not reported                              | NAS           |
| MIGS-4.4| Altitude                 | about 0 m, sea level                      | NAS           |

Evidence codes - 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 of the Gene Ontology project [31].

**Genome sequencing and annotation**

**Genome project history**

This organism was selected for sequencing on the basis of its phylogenetic position [32], and is part of the *Genomic Encyclopedia of Bacteria and Archaea* project [33]. The genome project is deposited in the Genomes On Line Database [22] and the complete genome sequence is deposited in 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                                                                                 |
| MIGS-28 | Libraries used               | Four genomic libraries: one 454 pyrosequence standard library, two 454 PE libraries (4 kb and 8 kb insert size), one Illumina library |
| MIGS-29 | Sequencing platforms         | Illumina GAii, 454 GS FLX Titanium                                                       |
| MIGS-30.2| Sequencing coverage         | 996.4 x Illumina; 36.4 x pyrosequence                                                   |
| MIGS-30 | Assemblers                   | Newbler version 2.3, Velvet version 0.7.63, phrap version SPS - 4.24                    |
| MIGS-32 | Gene calling method          | Prodigal 1.4, GenePRIMP                                                                  |
|         | INSDC ID                     | CP002999                                                                                 |
|         | Genbank Date of Release      | August 19, 2011                                                                          |
|         | GOLD ID                      | Gc01927                                                                                  |
|         | NCBI project ID              | 52467                                                                                    |
|         | Database: IMG-GEBA           | 2505679007                                                                               |
| MIGS-13 | Source material identifier   | DSM 13258                                                                               |
|         | Project relevance            | Tree of Life, GEBA                                                                        |

**Growth conditions and DNA isolation**

*M. rusestringensis* strain B13, DSM 13258, was grown in DSMZ medium 917 (Modified Sea Water Agar) [34] at 30°C. DNA was isolated from 0.5-1 g of cell paste using Jetflex Genomic DNA Purification Kit (GENOMED 600100) following the manufacturer’s instructions, with a modified procedure for cell lysis: incubation with 40 µl proteinase K for 40 min at 58°C. DNA is available through the DNA Bank Network [35].

**Genome sequencing and assembly**

The genome was sequenced using a combination of Illumina and 454 sequencing platforms. All general aspects of library construction and sequencing can be found at the JGI website [16]. Pyrosequencing reads were assembled using the Newbler assembler (Roche). The initial Newbler assembly consisting of 26 contigs in one scaffold was converted into a phrap [17] assembly by making fake reads from the consensus, to collect the read pairs in the 454 paired end library. Illumina GAii sequencing data (3,847 Mb) was assembled with Velvet [36] and the consensus sequences were shredded into 1.5 kb overlapped fake reads and assembled together with the 454 data. The 454 draft assembly was based on 268.3 Mb 454 draft data and all of the 454 paired end data. Newbler parameters are -consed -a 50 -l 350 -g -m -ml 20. The Phred/Phrap/Consed software package [17] was used for sequence assembly and quality assessment in the subsequent finishing process. After the shotgun stage, reads were assembled with parallel phrap (High Performance Software, LLC). Possible mis-assemblies were corrected with gapResolution [16], Dupfinisher [37], or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were closed by editing in Consed, by PCR and by Bubble PCR primer walks (J.F. Chang, unpublished). A total of 46 additional reactions were necessary to close gaps and to raise the quality of the finished sequence. Illumina reads were also used to correct potential base errors and increase consensus quality using a software Polisher developed at JGI [38]. The error rate of the completed genome sequence is less than 1 in 100,000. Together, the combination of the Illumina and 454 sequencing platforms provided 1,032.9 x coverage of the genome. The final assembly contained 422,407 pyrosequence and 49,819,141 Illumina reads.

**Genome annotation**
Genes were identified using Prodigal [39] as part of the Oak Ridge National Laboratory genome annotation pipeline, followed by a round of manual curation using the JGI GenePRIMP pipeline [40]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) nonredundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro databases. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes - Expert Review (IMG-ER) platform [41].

**Genome properties**

The genome consists of a 3,842,422 bp long circular chromosome with a G+C content of 41.4% (Table 3 and Figure 3). Of the 3,525 genes predicted, 3,478 were protein-coding genes, and 47 RNAs; 46 pseudogenes were also identified. The majority of the protein-coding genes (66.6%) were assigned with a putative function while the remaining ones were annotated as hypothetical proteins. The distribution of genes into COGs functional categories is presented in Table 4.

**Table 3. Genome Statistics**

| Attribute                        | Value  | % of Total |
|----------------------------------|--------|------------|
| Genome size (bp)                 | 3,842,422 | 100.00%    |
| DNA coding region (bp)           | 3,479,569 | 90.56%     |
| DNA G+C content (bp)             | 1,589,148 | 41.36%     |
| Number of replicons              | 1      |            |
| Extrachromosomal elements        | 0      |            |
| Total genes                      | 3,525  | 100.00%    |
| RNA genes                        | 47     | 1.33%      |
| rRNA operons                     | 2      |            |
| tRNA genes                       | 38     | 1.08%      |
| Protein-coding genes             | 3,478  | 98.67%     |
| Pseudo genes                     | 46     | 1.30%      |
| Genes with function prediction   | 2,349  | 66.64%     |
| Genes in paralog clusters        | 1,644  | 46.64%     |
| Genes assigned to COGs           | 2,433  | 69.02%     |
| Genes assigned Pfam domains      | 2,500  | 70.92%     |
| Genes with signal peptides       | 970    | 27.52%     |
| Genes with transmembrane helices | 809    | 22.95%     |
| CRISPR repeats                   | 0      |            |
Figure 3. Graphical map of the chromosome. 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 4. Number of genes associated with the general COG functional categories

| Code | COG counts and percentage of protein-coding genes | Description                                                                 |
|------|---------------------------------------------------|------------------------------------------------------------------------------|
| J    | 151                                               | Genome value 5.8 Translation, ribosomal structure and biogenesis             |
| A    | 0                                                 | Genome value 0.0 RNA processing and modification                             |
| K    | 206                                               | Genome value 7.9 Transcription                                               |
| L    | 130                                               | Genome value 5.0 Replication, recombination and repair                       |
| B    | 2                                                 | Genome value 0.1 Chromatin structure and dynamics                            |
| D    | 23                                                | Genome value 0.9 Cell cycle control, cell division, chromosome partitioning  |
| Y    | 0                                                 | Genome value 0.0 Nuclear structure                                           |
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References
1. Bruns A, Rohde M, Berthe-Corti L. *Muricauda ruestringensis* gen.nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* 2001; 51:1997-2006.
2. Yoon JH, Lee MH, Oh TK, Park YH. Muricauda flavescens sp. nov. and Muricauda aquimarina sp. nov., isolated from a salt lake near Hwajinpo Beach of the East Sea in Korea, and emended description of the genus Muricauda. *Int J Syst Evol Microbiol* 2005; 55:1015-1019.
3. Hwang CY, Kim MH, Bae GD, Zhang GI, Kim YH, Cho BC. Muricauda olearia sp. nov., isolated from crude-oil-contaminated seawater, and emended description of the genus Muricauda. *Int J Syst Evol Microbiol* 2009; 59:1856-1861.
4. Cavalier-Smith T. The neomuran origin of archaeabacteria, the negibacterial root of the universal tree and bacterial megaclassification. *Int J Syst Evol Microbiol* 2002; 52:7-76.
5. Ludwig W, Euzéby J, Whitman WG. Draft taxonomic outline of the Bacteroidetes, Planctomycetes, Chlamydiae, Spirochaetes, Fibrobacteres, Fusobacteria, Acidobacteria, Verrucomicrobia, Dictyoglomi, and Gemmatimonadetes.
Judicial Commission of the International Committee on Systematics of Prokaryotes. The nomenclatural types of the orders Acholeplasmatales, Halanaerobiales, Halobacterales, Methanobacterales, Methanococcales, Methanomicrobiales, Planctomycetales, Prochlorales, Sulfolobales, Thermococcales, Thermoproteales and Verrucomicrobiales are the genera Acholeplasma, Halanaerobium, Halobacterium, Methanobacterium, Methanococcus, Methanomicrobium, Planctomyces, Prochloron, Sulfolobus, Thermococcus, Thermoproteus and Verrucomicrobius, respectively. Opinion 79. *Int J Syst Evol Microbiol* 2005; **55**:517-518.

7. Altschul S, Gish W, Miller W, Myers E, Lipman D. Basic local alignment search tool. *J Mol Biol* 1990; **215**:403-410.

8. Korf I, Yandell M, Bedell J. BLAST, O'Reilly, Sebastopol, 2003.

9. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl Env Microbiol* 2006; **72**:5069-5072.

10. Porter MF. An algorithm for suffix stripping. *Program: electronic library and information systems* 1980; **14**:130-137.

11. Garrity GM, Holt JG. Taxonomic Outline of the Archaea and Bacteria. In: Garrity GM, Boone DR, Castenholz RW (eds), Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 1, Springer, New York, 2001, p. 155-166.

12. List Editor. Validation List No. 143. *Int J Syst Evol Microbiol* 2012; **62**:1-4.

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

14. Swofford DL. PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4.0 b10. Sinauer Associates, Sunderland, 2002.

15. Bernardet J-F. Order I. Flavobacteriales ord. nov. In: Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ, Ward NL, Ludwig W, Whitman WB (eds), Bergey's Manual of Systematic Bacteriology, Second Edition, Volume 4, Springer, New York, 2010, p. 105.

16. JGI website. [http://www.jgi.doe.gov/](http://www.jgi.doe.gov/).

17. The Phred/Phrap/Consed software package. [http://www.phrap.com](http://www.phrap.com).

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

19. Castresana J. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Mol Biol Evol* 2000; **17**:540-552.

20. Garrity G. NamesforLife. BrowserTool takes expertise out of the database and puts it right in the browser. *Microbiol Today* 2010; **37**:9.

21. Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A. How many bootstrap replicates are necessary? *Lect Notes in Comput Sci* 2009; **5541**:184-200.

22. Pagani I, Liolios K, Jansson J, Chen IM, Smirnova T, Nosrat B, Markowitz VM, Kyrpides NC. The Genomes OnLine Database (GOLD) v.4: status of genomic and metagenomic projects and their associated metadata. *Nucleic Acids Res* 2012; **40**:D571-D579.

23. Reichenbach H. Order 1. Cytophagales Leadbetter 1974, 99AL. In: Holt JG (ed), Bergey's Manual of Systematic Bacteriology, First Edition, Volume 3, The Williams and Wilkins Co., Baltimore, 1989, p. 2011-2013.
24. List Editor. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. List No. 41. *Int J Syst Bacteriol* 1992; 42:327-328.

25. Bernardet JF, Segers P, Vancanneyt M, Berthe F, Kersters K, Vandamme P. Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (Basonym, Cytophaga aquatilis Strohl and Tait 1978). *Int J Syst Bacteriol* 1992; 42:327-328.

26. Bernardet JF, Nakagawa Y, Holmes B. Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae*, and emended description of the family. *Int J Syst Evol Microbiol* 2002; 52:1049-1070.

27. 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.

28. 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.

29. Garrity GM, Lilburn TG, Cole JR, Harrison SH, Euzéby J, Tindall BJ. Taxonomic Outline of the Bacteria and Archaea, Release 7.7 March 6, 2007. Part 1 – The “Archaea”, Phyla “Crenarchaeota” and “Euryarchaeota”. *Taxonomic Outline 2007;*. doi:10.1601/toba7.7.

30. BAuA. Classification of bacteria and archaea in risk groups. TRBA 466. p. 141. Bundesanstalt für Arbeitsschutz und Arbeitsmedizin, Germany. 2010.

31. 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. *Nat Genet* 2000; 25:25-29.

32. Klenk H, Göker M. En route to a genome-based classification of *Archaea* and *Bacteria*? *Syst Appl Microbiol* 2010; 33:175-182.

33. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu M, Tindall BJ, et al. A phylogeny-driven genomic encyclopaedia of *Bacteria* and *Archaea*. *Nature* 2009; 462:1056-1060.

34. List of growth media used at DSMZ: http://www.dsmz.de/catalogues/catalogue-microorganisms/culture-technology/list-of-media-for-microorganisms.html.

35. Gemeinholzer B, Dröge G, Zetzsche H, Haszprunar G, Klenk HP, Güntsch A, Berendsohn WG, Wägele JW. The DNA Bank Network: the start from a German initiative. *Biopreserv Biobank* 2011; 9:51-55.

36. Zerbino DR, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. *Genome Res* 2008; 18:821-829.

37. Han C, Chain P. Finishing repeat regions automatically with Dupfinisher. *In: Proceeding of the 2006 international conference on bioinformatics & computational biology*. Arabnia HR, Valafar H (eds), CSREA Press. June 26-29, 2006: 141-146.

38. Lapidus A, LaButti K, Foster B, Lowry S, Trong S, Goltsman E. POLISHER: An effective tool for using ultra short reads in microbial genome assembly and finishing. *AGBT*, Marco Island, FL, 2008.

39. Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 2010; 11:119.

40. Pati A, Ivanova NN, Mikhailova N, Ovchinnikova G, Hooper SD, Lykidis A, Kyrpides NC. GenePRIMP: a gene prediction improvement pipeline for prokaryotic genomes. *Nat Methods* 2010; 7:455-457.
41. Markowitz VM, Ivanova NN, Chen I-MA, Chu K, Kyrpides NC. IMG ER: a system for microbial genome annotation expert review and curation. *Bioinformatics* 2009; **25**:2271-2278.
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