High quality draft genome sequence of the slightly halophilic bacterium *Halomonas zhanjiangensis* type strain JSM 078169T (DSM 21076T) from a sea urchin in southern China

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*Halomonas zhanjiangensis* Chen *et al*. 2009 is a member of the genus *Halomonas*, family *Halomonadaceae*, class Gammaproteobacteria. Representatives of the genus *Halomonas* are a group of halophilic bacteria often isolated from salty environments. The type strain *H. zhanjiangensis* JSM 078169T was isolated from a sea urchin (*Hemicentrotus pulcherrimus*) collected from the South China Sea. The genome of strain JSM 078169T is the fourteenth sequenced genome in the genus *Halomonas* and the fifteenth in the family *Halomonadaceae*. The other thirteen genomes from the genus *Halomonas* are *H. halocynthiae*, *H. venusta*, *H. alkaliphila*, *H. lutea*, *H. anticariensis*, *H. jeotgali*, *H. titanicae*, *H. desiderata*, *H. smyrnensis*, *H. salitiodae*, *H. boliviensis*, *H. elongata* and *H. stevensii*. Here, we describe the features of strain JSM 078169T, together with the complete genome sequence and annotation from a culture of DSM 21076T. The 4,060,520 bp long draft genome consists of 17 scaffolds with the 3,659 protein-coding and 80 RNA genes and is a part of *Genomic Encyclopedia of Type Strains*, Phase I: the one thousand microbial genomes (KMG) project.

**Keywords:** strictly aerobic, motile Gram-negative, chemoorganotrophic, slightly halophilic, *Halomonadaceae*

**Introduction**

Strain JSM 078169T (= DSM 21076 = KCTC 22279 = CCTCC AB 208031) is the type strain of the species *Halomonas zhanjiangensis* [1], one out of 84 species with a validly published name in the genus *Halomonas* [2], family *Halomonadaceae* [3]. The family *Halomonadaceae* currently comprises thirteen genera (*Aidingimonas*, *Carnimonas*, *Chromohalobacter*, *Cobetia*, *Halomonas*, *Halotalea*, *Halo-
vibrio, Kushneria, Marinspirillum, Modicisali-, bacter, Candiditus Portiera, Salinicola and Zymo-, bacter) with Halomonas being the largest genus in this family [3-6]. Members of the genus Halomo-, nas have been isolated from various saline en-,vironments and showed halophilic characteristics [7-11]. Strain JSM 078169T was originally isolated from a sea urchin (Hemicentrotus pulcher-rimus) that was collected from the South China Sea. The gen- us name was derived from the Greek words 'halos' meaning 'salt' and 'monas' meaning 'mon-, ad', yielding the Neo-Latin word 'halomonas' [2]; the species epithet was derived from Latin word 'zhanjiangensis', of Zhanjiang, a city in China near where the sample was collected [1]. Strain JSM 078169T was found to assimilate several mono-, and disaccharides and to produce numerous acid and alkaline phosphatases, leucine arylamidase, naphthol-ASBI-phosphohydrolase and valine arylamidase [1]. There are no PubMed records that document the use of these strain for any biotechnological studies; only comparative analyses performed for the description of later members of the genus Halomonas are recorded. However, the NamesforLife [12] database reports at least 70 pa-, tents in which Halomonas ssp. are referenced. Here we present a summary classification and a set of feature for H. zhanjiangensis JSM 078169T, together with the description of the genomic s-, equencing and annotation of DSM 21076.

Classification and features

16S rRNA analysis

The original assembly of the genome did not con-, tain longer stretches of 16S rRNA copies. There-, fore, a 1,413 bp long fragment of the 16S rRNA gene was later patched into the genome sequence assembly. This almost full length version of the 16S rRNA sequence was compared using NCBI BLAST [13,14] under default settings (e.g., considering only the high-scoring segment pairs (HSPs) from the best 250 hits) with the most recent ver-, sion of the Greengenes database [15] and the relative frequencies of taxa and unidentified clones (or strains) were calculated by BLAST scores. The most frequently occurring genus was Halomonas (74.8%), and the unidentified clones or isolates represented 25.5% for the total BLAST results. Ex-, cept for sequences of representatives of the genus Halomonas, no sequences from other genera were observed in the BLAST search. The highest degree of sequence similarity was reported with H. alka-, ntarctica str. CRSS.

Figure 1 shows the phylogenetic neighborhood of H. zhanjiangensis JSM 078169T in a tree based on 16S rRNA genes. The 1,413 bp long sequence fragment of the 16S rRNA gene differs by three nucleotides from the previously published 16S rRNA sequence (FJ429198). The tree provided a precise insight into the nomenclature and classifi-, cation of members of the genus Halomonas. The phylogenetic analysis showed that strain H. zhanjiangensis JSM 078169T was most closely re-, lated to H. nanhaiensis YIM M 13059T with 98.3% sequence similarity.

Morphology and physiology

H. zhanjiangensis JSM 078169T is a Gram-negative-staining, non-spore-forming, strictly aerobic (Table 1), catalase-positive, oxidase-negative and slightly halophilic bacterium that reduces nitrate [1]. Cells of JSM 078169T are short rods (0.4-0.7 μm × 0.6-1.0 μm) and motile with peritrichous flagella (not visible in Figure 2). Colonies are yellow-, pigmented, flat and non-translucent with glisten-, ing surfaces and circular/slightly irregular mar-, gins, 2-3 mm in diameter after incubation on Ma-, rine Agar (MA) at 28 ºC for 3-5 days. No diffusible pigments are produced. Growth occurs at 4-40 ºC with an optimum growth at 25-30 ºC, at pH range of 6.0-10.5 with an optimum pH of 7.5. The salinity range suitable for growth was 1.0-20.0% (w/v) total salts with an optimum between 3.0-5.0% (w/v) total salts. No growth occurs in the absence of NaCl or with NaCl as the sole salt. Strain JSM 078169T grows on Marine Agar and the medium contained the following: 5.0 g peptone, 1.0 g yeast extract, 0.1 g ferric citrate, 19.45 g NaCl, 8.8 g MgCl2, 3.24 g Na2SO4, 1.8 g CaCl2, 0.55 g KCl, 0.16 g NaHCO3, 0.08 g KBr, 0.034 g SrCl2, 0.022 g H3BO3, 0.004 g sodium silicate, 0.0024 g sodium fluoride, 0.0016 g ammonium nitrate, 0.008 g disodium phosphate and 15 g agar.
Figure 1. Phylogenetic tree highlighting the position of *H. zhanjiangensis* relative to the closest related type strains of the other species within the family *Halomonadaceae*. All the 16S rRNA gene sequences of the type strains within the genus *Halomonas* were included and combined with the representative 16S rRNA gene sequences of the type species in other genera, according to the most recent release of the EzTaxon database. The tree was inferred from 1,381 aligned characters [16] under the neighbor-joining (NJ) [17], and maximum-likelihood (ML) [18] method with 1,000 randomly selected bootstrap replicates using MEGA version 5.2 [19]. The branches are scaled in terms of the expected number of substitutions per site. Numbers adjacent to the branches are support values from 1,000 NJ bootstrap (left) and from 1,000 ML bootstrap (right) replicates [20] if larger than 60%. Lineages with type strain genome sequencing projects registered in GOLD [21] are labeled with one asterisk, those also listed as ‘Complete and Published’ with two asterisks [22].
Table 1 Classification and general features of *H. zhanjiangensis* JSM 078169\(^1\) according the MIGS recommendations [23], (published by the Genomic Standards Consortium [24]), List of Prokaryotic names with Standing in Nomenclature [25] and the Names for Life database [12].

| MIGS ID | Property       | Term                          | Evidence code |
|---------|----------------|-------------------------------|---------------|
| MIGS-2  | Domain         | Bacteria                      | TAS [26]      |
|         | Phylum         | Proteobacteria                | TAS [27]      |
|         | Class          | Gammaproteobacteria           | TAS [28-30]   |
|         | Order          | Oceanospirillales             | TAS [29,31]   |
| MIGS-3  | Family         | Halomonadaceae                | TAS [3,32-35] |
|         | Genus          | Halomonas                     | TAS [2,36]    |
|         | Species        | Halomonas zhanjiangensis      | TAS [1]       |
| MIGS-37.1 | Type strain  | JSM 078169                   | TAS [1]       |
| MIGS-37.2 | Cell shape    | rod-shaped                    | TAS [1]       |
| MIGS-37.3 | Motility      | motile                        | TAS [1]       |
| MIGS-37.4 | Sporulation   | non-sporulating               | TAS [1]       |
| MIGS-37.12 | Temperature | 4-40°C                        | TAS [1]       |
|         | Optimum temperature | 25-30°C             | TAS [1]       |
|         | Salinity       | 1-20% NaCl (w/v), optimum 3-5% | TAS [1]       |
|         | pH             | 6.0-10.5                      | TAS [1]       |
| MIGS-37.5 | Cell diameter | 0.7-1.4 μm                   | TAS [1]       |
| MIGS-37.6 | Cell length   | 1.5-2.5 μm                   | TAS [1]       |
| MIGS-37.9 | Cell arrangement | singles, pairs          | TAS [1]       |
| MIGS-37.11 | Energy metabolism | chemoorganotrophic      | TAS [1]       |
| MIGS-6   | Habitat        | aquatic, marine host         | TAS [1]       |
| MIGS-22  | Oxygen requirement | aerobe                    | TAS [1]       |
| MIGS-15  | Biotic relationship | not reported             | TAS [1]       |
| MIGS-16  | Host name      | *Hemicentrotus pulcherrimus* (sea urchin) | TAS [1]       |
| MIGS-4.5 | Isolation site | Unspecified                  | TAS [1]       |
|         | Geographic location | Zhanjiang            | TAS [1]       |
| MIGS-4.1 | Latitude       | 20.90                        | TAS [1]       |
| MIGS-4.2 | Longitude      | 110.59                       | TAS [1]       |
| MIGS-4.3 | Altitude       | not reported                 |               |
| MIGS-4.4 | Depth          | not reported                 |               |

Evidence codes-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). Evidence codes are from the Gene Ontology project [37].
**Halomonas zhanjiangensis**

**Chemotaxonomy**
The predominant respiratory quinone is Q-9 which is consistent to the other members of the genus *Halomonas* [1]. The predominant fatty acids are C_{18:1}ω7c (48.9%), C_{16:0} (17.0%) and C_{12:0} 3-OH (10.7%). The profile of major fatty acids is also similar to the other representatives of the genus *Halomonas* [38-41].

**Genome sequencing and annotation**

**Genome project history**
This organism was selected for sequencing on the basis of its phylogenetic position [42,43]. Sequencing and library preparation artifacts [51]. Following steps were then performed for assembly: (1) filtered Illumina reads were assembled using Velvet [52], (2) 1–3 kbp simulated paired end reads were created from Velvet contigs using wgsim [53], (3) Illumina reads were assembled with simulated read pairs using Allpaths–LG [54]. Parameters for assembly steps were: 1) Velvet (velveth: 63 –shortPaired and velvetg: –very clean yes –export-Filtered yes –min contig lgth 500 – scaffolding no –cov cutoff 10) 2) wgsim (–e 0 –1 100 –2 100 –r 0 –R 0 –X 0) 3) Allpaths–LG (PrepareAllpathsInputs: PHRED 64=1 PLOIDY=1 FRAG COVERAGE=125 JUMP COVERAGE=25 LONG JUMP COV=50, RunAllpathsLG: THREADS=8 RUN=std shreads TARGETS=standard VAPI WARN ONLY=True OVERWRITE=True). The final draft assembly contained 18 contigs in 17 scaffolds. The total size of the genome is 4.1 Mbp and the final assembly is based on 501.3 Mbp of Illumina data, which provides an average 123.5 × coverage of the genome.

**Growth conditions and DNA isolation**
*H. zhanjiangensis* JSM 078169^T^, DSM 21076, was grown in DSMZ medium 1510 (modified medium 514 for *Halomonas* sp.) [47] at 28 °C. DNA was isolated from 0.5-1.0 g of cell paste using MasterPure Gram-positive DNA purification kit (Epicentre MGP04100) following the standard protocol as recommended by the manufacturer with modification st/DL for cell lysis as described by Wu et al. [45]. DNA is available through the DNA Bank Network [48].

**Genome sequencing and assembly**
The draft genome sequence was generated using the Illumina technology [49]. An Illumina Standard shotgun library was constructed and sequenced using the Illumina HiSeq 2000 platform which generated 15,593,002 reads totaling 2,339.0 Mbp. All general aspects of library construction and sequencing performed at the JGI can be found at [50]. All raw Illumina sequence data was passed through DUK, a filtering program developed at JGI, which removes known Illumina sequencing and library preparation artifacts [51].

| MIGS ID | Property                      | Term                                      |
|---------|-------------------------------|-------------------------------------------|
| MIGS-31.1 | Sequencing quality           | Level 2: High-Quality Draft              |
| MIGS-28.1 | Libraries used               | One Illumina standard shotgun library    |
| MIGS-29  | Sequencing method            | Illumina HiSeq 2000,                     |
|          | Assembly method              | Velvet v. 1.1.04; ALLPATHS v. r41043     |
|          | Gene calling method          | Prodigal                                  |
| MIGS-30  | INSDC ID                     | ARIT00000000                              |
| MIGS-32  | Genbank date of release      | December 12, 2013                         |
|          | GOLD ID                      | Gi11554                                   |
| MIGS-1.1 | NCBI project ID              | 178047                                    |
| MIGS-1.2 | Straininfo ID                | 845770                                    |
|          | Database: IMG                | 2517572236                                |
| MIGS-13  | Source material identifier   | DSM 21076                                 |
| MIGS-38.2 | Project relevance            | Tree of Life, GEBA-KMG                   |

**Table 2 Genome sequencing project information**
Genome annotation
Genes were identified using Prodigal [55] as part of the DOE-JGI genome annotation pipeline [56], following by a round of manual curation using the JGI GenePRIMP pipeline [57]. The predicted CDSs were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGR-Fam, Pfam, PRIAM, KEGG, COG, and InterPro database. These data sources were combined to assert a product description for each predicted protein. Additional gene prediction analysis and functional annotation was performed within the Integrated Microbial Genomes-Expert Review (IMG-ER) platform [58].

Genome properties
The assembly of the draft genome sequence consists of 17 scaffolds amounting to 4,060,520 bp, and the G+C content is 54.5% (Table 3 and Figure 3). Of the 3,739 genes predicted, 3,659 were protein-coding genes, and 80 RNAs. The majority of the protein-coding genes (87.1%) were assigned 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 and Figure 3.

Table 3. Genome statistics

| Attribute                                         | Value         | % of total |
|---------------------------------------------------|---------------|------------|
| Genome size (bp)                                  | 4,060,520     | 100.00%    |
| DNA coding region (bp)                            | 3,661,597     | 90.18%     |
| DNA G+C content (bp)                              | 2,212,212     | 54.48%     |
| Number of scaffolds                               | 17            |            |
| Extrachromosomal elements                         | unknown       |            |
| Total genes                                       | 3,739         | 100.00%    |
| RNA genes                                         | 80            | 2.14%      |
| rRNA operons                                      | unknown       |            |
| tRNA genes                                        | 56            | 1.50%      |
| Protein-coding genes                              | 3,659         | 97.86%     |
| Pseudo genes                                      | 0             | 0.00%      |
| Genes with function prediction (proteins)         | 3,256         | 87.08%     |
| Genes in paralog clusters                         | 2,856         | 76.38%     |
| Genes assigned to COGs                            | 3,175         | 84.92%     |
| Genes assigned Pfam domains                       | 3,303         | 88.34%     |
| Genes with signal peptides                        | 313           | 8.37%      |
| Genes with transmembrane helices                  | 941           | 25.17%     |
| CRISPR repeats                                    | 1             |            |

Figure 3 The graphical map of the largest scaffold of the genome. From bottom to the top: Genes on forward strand (color by COG categories), Genes on reverse strand (color by COG categories), RNA genes (tRNA green, rRNA red, other RNAs black), GC content, GC skew (purple/olive).
Insights into the genome sequence

One complete genome sequence from a type strain of the family *Halomonas* - *H. elongata* [22] is available in GenBank, and four other permanent draft genomes of *H. anticariensis*, *H. lutea*, *H. jeotgali* and *H. halocynthiae* are available from IMG. The genome size of *H. zhanjiangensis* is smaller than those of *H. elongata*, *H. lutea* and *H. anticariensis* (4.06-5.02 Mbp), but much larger than those of *H. jeotgali* and *H. halocynthiae* (2.85-2.88 Mbp). Using the genome-to-genome distance calculator [59-61] version 2.0 revealed that all digital DNA-DNA hybridization (DDH) values are much lower than 70% using the program NCBI-BLAST, which demonstrated that *H. zhanjiangensis* is distinct from *H. elongata*, *H. anticariensis*, *H. lutea*, *H. jeotgali* and *H. halocynthiae* at the species level. Distance is 0.1845 between the type strain genomes of *H. zhanjiangensis* and *H. elongata*, which corresponds to a DDH value of 13.00 ± 2.99%. The distances of *H. zhanjiangensis* from *H. anticariensis*, *H. lutea*, *H. jeotgali* and *H. halocynthiae* are 0.1842, 0.1837, 0.1835 and 0.1849, which correspond to DDH values of 20.30 ± 2.41%, 20.30 ± 2.41%, 20.40 ± 2.41% and 20.40 ± 2.41%, respectively.

A major feature of the previously sequenced genomes from this family is the presence of large numbers of proteins for the TRAP-type C4-dicarboxylate transport systems. A total of 267 genes in the genome of *H. zhanjiangensis* encode proteins for carbohydrate transport and metabolism, 68 genes are related to TRAP-type C4-dicarboxylate transport systems and encoded 22 large permease proteins, 24 periplasmic proteins and 22 small permease proteins. Genomic analysis of *H. elongata*, *H. anticariensis*, *H. lutea*, *H. jeotgali* and *H. halocynthiae* showed that they encode 58, 65, 61, 7 and 32 proteins related to TRAP-type C4-dicarboxylate transport systems and encoded 22 large permease proteins, 24 periplasmic proteins and 22 small permease proteins. Genomic analysis of *H. elongata*, *H. anticariensis*, *H. lutea*, *H. jeotgali* and *H. halocynthiae* showed that they encode 58, 65, 61, 7 and 32 proteins related to TRAP-type C4-dicarboxylate transport systems respectively. Proteins for TRAP-type C4-dicarboxylate transport systems constitute 1.86% as the total protein-coding sequences of the *H. zhanjiangensis* genome. In the genomes of *H. elongata*, *H. anticariensis*, *H. lutea*, *H. jeotgali* and *H. halocynthiae*, TRAP-type
C4-dicarboxylate transport system related proteins are accounted for 1.67%, 1.37%, 1.42%, 0.27% and 1.18% of the total protein-coding genes respectively. Therefore, *H. zhanjiangensis* has the highest percentage of TRAP-type C4-dicarboxylate transport system related encoding proteins in this group of bacteria to date.

Of the signal transduction mechanisms, Methyl-accepting Chemotaxis Proteins (MCPs) are transmembrane sensor proteins of bacteria. The MCPs allow bacteria to detect concentrations of molecules in the extracellular matrix so that they may smoothly swim or tumble accordingly [62,63]. Various environmental conditions give rise to diversity in bacterial signaling receptors, and consequently there are many genes encoding MCPs [64]. A number of MCPs (23) are present in *H. zhanjiangensis*, while *H. elongata*, *H. anticariensis*, *H. lutea*, and *H. jeotgali* have only 4, 21, 16, and 17 MCPs, respectively. MCPs are not found in the genome of *H. halocynthiae*. *H. zhanjiangensis* has the largest numbers of MCPs in this family. The analysis of bacterial genomes reveals that the family *Halomonadaceae* differs enormously in the number of MCPs from *E. coli*, and the number of MCPs in *Halomonadaceae* is about two times than that of *E. coli* strains.

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**References**

1. Chen YG, Zhang YQ, Huang HY, Klenk HP, Tang SK, Huang K, Chen QH, Cui XL, Li WJ. *Halomonas zhanjiangensis* sp. nov., a halophilic bacterium isolated from a sea urchin. *Int J Syst Evol Microbiol* 2009; 59:2888-2893. PubMed [http://dx.doi.org/10.1099/ijs.0.010173-0](http://dx.doi.org/10.1099/ijs.0.010173-0)

2. Vreeland R, Litchfield CD, Martin EL, Elliot E. *Halomonas elongata*, a new genus and species of extremely salt-tolerant bacteria. *Int J Syst Evol Microbiol* 1980; 30:485-495.

3. Franzmann PD, Wehmeyer U, Stackebrandt E. *Halomonadaceae* fam. nov., a new family of the class Proteobacteria to accommodate the genera *Halomonas* and *Deleya*. *Syst Appl Microbiol* 1988; 11:16-19. [http://dx.doi.org/10.1016/S0723-2020(88)80043-2](http://dx.doi.org/10.1016/S0723-2020(88)80043-2)

4. Anzai Y, Kim H, Park JY, Wakabayashi H, Oyaizu H. Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* 2000; 50:1563-1589. PubMed [http://dx.doi.org/10.1099/00277135-50-4-1563](http://dx.doi.org/10.1099/00277135-50-4-1563)

5. Arahal DR, Ludwig W, Schleifer KH, Ventosa A. Phylogeny of the family *Halomonadaceae* based on 235 and 16S rDNA sequence analyses. *Int J Syst Evol Microbiol* 2002; 52:241-249. PubMed [http://dx.doi.org/10.1099/00277135-50-4-1563](http://dx.doi.org/10.1099/00277135-50-4-1563)

6. Mellado E, Moore ERB, Nieto JJ, Ventosa A. Phylogenetic inferences and taxonomic consequences of 16S ribosomal DNA sequence comparison of *Chromohalobacter marismortui*, *Volcaniella eurihalina*, and *Deleya salina* and reclassification of *V. eurihalina* as *Halomonas eurihalina* comb. nov. *Int J Syst Evol Microbiol* 1995; 45:712-716. PubMed [http://dx.doi.org/10.1099/00277135-50-4-1563](http://dx.doi.org/10.1099/00277135-50-4-1563)

7. Cabrera A, Aguilera M, Fuentes S, Incerti C, Russell NJ, Ramos-Cormenzana A, Monteoliva-Sánchez M. *Halomonas indalinina* sp. nov., a moderately halophilic bacterium isolated from a solar saltern in Cabo de Gata, Almería, southern Spain. *Int J Syst Evol Microbiol* 2007; 57:376-380. PubMed [http://dx.doi.org/10.1099/00277135-50-4-1563](http://dx.doi.org/10.1099/00277135-50-4-1563)

8. Kim KK, Jin L, Yang HC, Lee ST. *Halomonas gomseomensis* sp. nov., *Halomonas janggokensis* sp. nov., *Halomonas salaria* sp. nov. and *Halomonas denitrificans* sp. nov., moderately halophilic bacteria isolated from saline water. *Int J Syst Evol Microbiol* 2007; 57:675-681. PubMed [http://dx.doi.org/10.1099/00277135-50-4-1563](http://dx.doi.org/10.1099/00277135-50-4-1563)

9. Soto-Ramírez N, Sánchez-Porro C, Rosas S, González W, Quiñones M, Ventosa A, Montalvo-Rodríguez R. *Halomonas avicenniae* sp. nov., isolated from the salty leaves of the black mangrove.

http://standardsingenomics.org
Halomonas zhanjiangensis

Avicennia germinans in Puerto Rico. *Int J Syst Evol Microbiol* 2007; **57**:900-905. PubMed http://dx.doi.org/10.1099/ijs.0.64818-0

10. Ventosa J, Nieto, Oren A. Biology of moderately halophilic aerobic bacteria. *Microbiol Mol Biol Rev* 1998; **62**:504-544. PubMed

11. Wang YN, Cai H, Chi CQ, Lu AH, Lin XG, Jiang ZF, Wu XL. *Halomonas shengliensis* sp. nov., a moderately halophilic, denitrifying, crude-oil-utilizing bacterium. *Int J Syst Evol Microbiol* 2007; **57**:1222-1226. PubMed http://dx.doi.org/10.1099/ijs.0.64973-0

12. Garrity GM. Names for Life Browser Tool takes expertise out of the database and puts it right in the browser. *Microbiol Today* 2010; **37**:9.

13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990; **215**:403-410. PubMed http://dx.doi.org/10.1016/S0022-2836(05)80360-2

14. Korf I, Yandell M, Bedell J. BLAST, O’Reilly, Sebastopol, 2003.

15. 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 Environ Microbiol* 2006; **72**:5069-5072. PubMed http://dx.doi.org/10.1128/AEM.03006-05

16. Lee C, Grasso C, Sharlow MF. Multiple sequence alignment using partial order graphs. *Bioinformatics* 2002; **18**:452-464. PubMed http://dx.doi.org/10.1093/bioinformatics/18.3.452

17. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987; **4**:406-425. PubMed

18. Strimmer K, von Haeseler A. Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. *Mol Biol Evol* 1996; **13**:964-969. http://dx.doi.org/10.1093/oxfordjournals.molbev.a025664

19. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; **28**:2731-2739. PubMed http://dx.doi.org/10.1093/molbev/msr121

20. Pattengale ND, Alipour M, Bininda-Emonds OR, Moret BM, Stamatakis A. How many bootstrap replicates are necessary? [doi:10.1089/cmb.2009.0179]. [pmid:20377449]. *J Comput Biol* 2010; **17**:337-354.

21. 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. PubMed http://dx.doi.org/10.1093/nar/gkr1100

22. Schwibbert K, Marin-Sanguino A, Bagyan I, Heidrich G, Lentzen G, Seitz H, Rampp M, Schuster SC, Klenk HP, Pfeiffer F, et al. A blueprint of ectoine metabolism from the genome of the industrial producer *Halomonas elongata* DSM 2581T. *Environ Microbiol* 2011; **13**:1973-1994. PubMed http://dx.doi.org/10.1111/j.1462-2920.2010.02336.x

23. 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

24. Field D, Amaral-Zettler L, Cochrane G, Cole JR, Dawyndt P, Garrity GM, Gilbert J, Glöckner FO, Hirschman L, Karsch-Mizrachi I, et al. *PLoS Biol* 2011; **9**:e1001088. PubMed http://dx.doi.org/10.1371/journal.pbio.1001088

25. Euzéby JP. List of bacterial names with standing in nomenclature: A folder available on the Internet. *Int J Syst Bacteriol* 1997; **47**:590-592. PubMed http://dx.doi.org/10.1099/0022-2990-47-2-590

26. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domain Archaea. *J Bacteriol* 1990; **173**:5628-5633. PubMed http://dx.doi.org/10.1128/JB.173.13.5628-5633.90

27. Garrity GM, Lilburn T. Phylum XIV. *Proteobacteria* phyl. nov. In: Garrity GM, Krieg NR, Staley JT (eds), Bergey’s Manual of Systematic Bacteriology, Second Edition, Springer, New York, 2005.

28. Garrity GM, Bell JA, Lilburn T. Class III. *Gammaproteobacteria* class. nov. In: Brenner DJ, Krieg NR, Staley JT and Garrity GM (eds), Bergey’s Manual of Systematic Bacteriology, Second Edition, Volume 2, Part C, Springer, New York, 2005, p. 1.

29. Validation of publication of new names and new combinations previously effectively published outside the IJSEM. List no. 106. *Int J Syst Evol Microbiol*.
(Vreeland et al. 1980), Dobson SJ, Franzmann PD. Unification of the class 

 binnen der Gammaproteobacteria. Int J Syst Evol Microbiol 2013; 63:2901-2906. PubMed http://dx.doi.org/10.1099/ijs.0.049270-0

31. Garrity GM, Bell JA, Lilburn T. Order VIII. Oceanospirillales ord. nov. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds), Bergey's Manual of Systematic Bacteriology, second edition, vol. 2 (The Proteobacteria), part B (The Gammaproteobacteria), Springer, New York, 2005, p. 270.

32. Validation List no. 29. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. Int J Syst Bacteriol 1989; 39:205-206. http://dx.doi.org/10.1099/00207713-39-2-205

33. Dobson SJ, Franzmann PD. Unification of the genera Deleya (Baumann et al. 1983), Halomonas (Vreeland et al. 1980), and Halovibrio (Fendrich 1988) and the species Paracoccus halodenitrificans (Robinson and Gibbons 1952) into a single genus, Halomonas, and placement of the genus Zymobacter in the family Halomonadaceae. Int J Syst Bacteriol 1996; 46:550-558. http://dx.doi.org/10.1099/0207713-46-2-550

34. Ntougias S, Zervakis GI, Fasseas C. Halotalea alkalitolerans gen. nov., sp. nov., a novel osmotolerant and alkalitolerant bacterium from alkaline olive mill wastes, and emended description of the family Halomonadaceae Franzmann et al. 1989, emend. Dobson and Franzmann 1996. Int J Syst Evol Microbiol 2007; 57:1975-1983. PubMed http://dx.doi.org/10.1099/ijs.0.65007-0

35. Ben Ali Gam Z, Abdelkafi S, Casalot L, Tholozan JL, Oueslati R, Labat M. Medicsalbacillus tunisiensis gen. nov., sp. nov., an aerobic, moderately halophilic bacterium isolated from an oilfield-water injection sample, and emended description of the family Halomonadaceae Franzmann et al. 1989 emend. Dobson and Franzmann 1996 emend. Ntougias et al. 2007. Int J Syst Evol Microbiol 2007; 57:2307-2313. PubMed http://dx.doi.org/10.1099/ijs.0.65008-0

36. Dobson SJ, Franzmann PD. Unification of the genera Deleya (Baumann et al. 1983), Halomonas (Vreeland et al. 1980), and Halovibrio (Fendrich 1988) and the species Paracoccus halodenitrificans (Robinson and Gibbons 1952) into a single genus, Halomonas, and placement of the genus Zymobacter in the family Halomonadaceae. Int J Syst Bacteriol 1996; 46:550-558. http://dx.doi.org/10.1099/00207713-46-2-550

37. 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; 25:29-25. PubMed http://dx.doi.org/10.1038/75556

38. Berendes F, Gottschalk G, Heine-Dobbernack E, Moore ERB, Tindall BJ. Halomonas desiderata sp. nov, a new alkaliphilic, halotolerant and denitrifying bacterium isolated from a municipal sewage works. Syst Appl Microbiol 1996; 19:158-167. http://dx.doi.org/10.1016/S0723-2901(96)80041-5

39. Romano I, Gottschalk G, Heine-Dobbernack E, Moore ERB, Tindall BJ. Characterization of a haloalkaliphilic strictly aerobic bacterium, isolated from Pantelleria island. Syst Appl Microbiol 1996; 19:326-333. http://dx.doi.org/10.1016/S0723-2901(96)80059-2

40. Heyman J, Balcaen A, De Vos P, Swings J. Halomonas muralis sp. nov., isolated from microbial biofilms colonizing the walls and murals of the Saint-Catherine chapel (Castle Herberstein, Austria). Int J Syst Evol Microbiol 2002; 52:2049-2054. PubMed http://dx.doi.org/10.1099/ijs.0.02166-0

41. Jeon CO, Lim JM, Lee JR, Lee GS, Park DJ, Lee JC, Oh HW, Kim CJ. Halomonas kribbensis sp. nov., a novel moderately halophilic bacterium isolated from a solar saltern in Korea. Int J Syst Evol Microbiol 2007; 57:2194-2198. PubMed http://dx.doi.org/10.1099/ijs.0.65285-0

42. Klenk HP, Göker M. En route to a genome - based classification of Archaea and Bacteria? Syst Appl Microbiol 2010; 33:175-182. PubMed http://dx.doi.org/10.1016/j.syapm.2010.03.003

43. Göker M, Klenk HP. Phylogeny-driven target selection for genome-sequencing (and other) projects. Stand Genomic Sci 2013; 8:360-374. PubMed http://dx.doi.org/10.4056/sigs.3446951

44. Kyprides NC, Woyke T, Eisen JA, Garrity G, Lilburn TG, Beck BJ, Whitman WB, Hugenholtz P, Klenk HP. Genomic Encyclopedia of Type Strains, Phase I: the one thousand microbial genomes (KMG-I) project. Stand Genomic Sci 2013; 9:628-634. http://dx.doi.org/10.4056/sigs.5068949

45. Wu D, Hugenholtz P, Mavromatis K, Pukall R, Dalin E, Ivanova NN, Kunin V, Goodwin L, Wu

http://standardsingenomics.org
Halomonas zhanjiangensis

M, Tindall BJ, et al. A phylogeny-driven genomic encyclopaedia of Bacteria and Archaea. Nature 2009; 462:1056-1060. PubMed
http://dx.doi.org/10.1038/nature08656

46. Mavromatis K, Land ML, Brettin TS, Quest DJ, Copeland A, Clum A, Goodwin L, Woyke T, Lapidus A, Klenk HP, et al. The fast changing landscape of sequencing technologies and their impact on microbial genome assemblies and annotation. PLoS ONE 2012; 7:e48837. PubMed
http://dx.doi.org/10.1371/journal.pone.0048837

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

48. 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.
http://dx.doi.org/10.1089/bio.2010.0029

49. Bennett S. Solexa Ltd. Pharmacogenomics 2004; 5:433-438. PubMed
http://dx.doi.org/10.1517/14622416.5.4.433

50. The DOE Joint Genome Institute.
http://www.jgi.doe.gov

51. Mingkun L, Copeland A, Han J. DUK, unpublished, 2011.

52. Zerbino D, Birney E. Velvet: algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 2008; 18:821-829. PubMed
http://dx.doi.org/10.1101/gr.074492.107

53. https://github.com/lh3/wgsim

54. Gnerre S, MacCallum I. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proc Natl Acad Sci USA 2011; 108:1513-1518. PubMed
http://dx.doi.org/10.1073/pnas.1017351108

55. Hyatt D, Chen GL, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 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

56. Mavromatis K, Ivanova NN, Chen IM, Szeto E, Markowitz VM, Kyrpides NC. The DOE-JGI Standard operating procedure for the annotations of microbial genomes. Stand Genomic Sci 2009; 1:63-67. PubMed
http://dx.doi.org/10.4056/sigs.632

57. 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. PubMed
http://dx.doi.org/10.1038/nmeth.1457

58. Markowitz VM, Ivanova NN, Chen IMA, Chu K, Kyrpides 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

59. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC Bioinformatics 2013; 14:60. PubMed
http://dx.doi.org/10.1186/1471-2105-14-60

60. Auch AF, von Jan M, Klenk HP, Göker M. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Stand Genomic Sci 2010; 2:117-134. PubMed
http://dx.doi.org/10.4056/sigs.531120

61. Auch AF, Klenk HP, Göker M. Standard operating procedure for calculating genome-to-genome distances based on high-scoring segment pairs. Stand Genomic Sci 2010; 2:142-148. PubMed
http://dx.doi.org/10.4056/sigs.541628

62. Derr P, Boder E, Goulian M. Changing the specificity of a bacterial chemoreceptor. J Mol Biol 2006; 355:923-932. PubMed
http://dx.doi.org/10.1016/j.jmb.2005.11.025

63. Alexander RP, Zhulin IB. Evolutionary genomics reveals conserved structural determinants of signaling and adaptation in microbial chemoreceptors. Proc Natl Acad Sci USA 2007; 104:2885-2890. PubMed
http://dx.doi.org/10.1073/pnas.0609359104

64. Harrison DM, Skidmore J, Armitage JP, Maddock JR. Localization and environmental regulation of MCP-like proteins in Rhodobacter sphaeroides. Mol Microbiol 1999; 31:885-892. PubMed
http://dx.doi.org/10.1046/j.1365-2958.1999.01226.x