

8-2013

Mesoaciditoga lauensis gen. nov., sp. nov., a moderate thermoacidophilic Thermotogales from a deep-sea hydrothermal vent

Anna-Louise Reysenbach
Portland State University, reysenbacha@pdx.edu

Yitai Liu
Portland State University

Annie R. Lindgren
Portland State University, annie.lindgren@pdx.edu

Issac D. Wagner
Portland State University

Christine Demko Sislak
Portland State University, cdsislak@gmail.com

See next page for additional authors

Let us know how access to this document benefits you.

Follow this and additional works at: https://pdxscholar.library.pdx.edu/bio_fac

 Part of the [Microbiology Commons](#)

Citation Details

Reysenbach, A. L., Y. Liu, A. R. Lindgren, I. D. Wagner, C. D. Sislak, A. Mets, and S. Schouten. "Mesoaciditoga lauensis gen. nov., sp. nov., a moderate thermoacidophilic Thermotogales from a deep-sea hydrothermal vent." *International journal of systematic and evolutionary microbiology* (2013).

This Post-Print is brought to you for free and open access. It has been accepted for inclusion in Biology Faculty Publications and Presentations by an authorized administrator of PDXScholar. For more information, please contact pdxscholar@pdx.edu.

Authors

Anna-Louise Reysenbach, Yitai Liu, Annie R. Lindgren, Issac D. Wagner, Christine Demko Sislak, Annelique Mets, and Stefan Schouten

1 ***Mesoaciditoga lauensis* gen. nov., sp. nov., a moderate thermoacidophilic *Thermotogales***
2 **from a deep-sea hydrothermal vent.**

3

4

5 Anna-Louise Reysenbach^{1*}, Yitai Liu¹, Annie R. Lindgren¹, Isaac D. Wagner¹, Christine D.
6 Sislak¹, Anhelique Mets² and Stefan Schouten²

7

8 ¹Department of Biology and Center for Life in Extreme Environments, Portland State University,
9 Portland, OR 97201, USA

10 ²Royal Netherlands Institute for Sea Research, Department of Marine Organic Biogeochemistry,
11 1790 AB Den Burg, Texel, The Netherlands

12

13

14 *Corresponding author: Anna-Louise Reysenbach

15 Email: reysenbacha@pdx.edu

16 Phone: (503) 725 3864. Fax (503) 725 3888

17

18

19 Keywords: thermophile, acidophile, *Thermotogales*, deep-sea vents

20 Other Bacteria

21 Running title: Moderate thermoacidophilic *Thermotogales*, *Mesoaciditoga lauensis*

22 The GenBank accession number for the 16S rRNA gene sequence of strain cd-1655R is

23 JQ347593.

24 **Summary**

25 A novel moderately thermophilic, heterotrophic bacterium was isolated from a deep-sea
26 hydrothermal vent deposit from the Mariner field along the Eastern Lau Spreading Center,
27 Southwestern Pacific. Cells were short motile rods (about 0.4 μm -0.8 μm) that occurred singly or
28 in pairs and were surrounded by a sheath-like membrane or 'toga'. The cells grew between 45
29 and 65°C (optimum 57-60°C), pH 4.1-6.0 (optimum pH 5.5-5.7) and optimally at 3% (w/v)
30 NaCl. The isolate grew on a range of carbon and proteinaceous substrates and reduced sulfur.
31 The G + C content of the DNA was about 45 mol%. Phylogenetic analysis of the 16S rRNA
32 gene placed the new isolate as a deeply diverging lineage within the *Thermotogales*. Based on
33 the physiological, morphological and phylogenetic data, the isolate is a novel species of a new
34 genus with the proposed name *Mesoaciditoga lauensis* gen. nov. sp. nov. The type strain is cd-
35 1655R^T (DSM 25116, OCM 1212).

36

37

38

39 Members of the *Thermotogales* are generally extreme thermophiles (growing best above 80°C)
40 or moderate thermophiles growing best around 65°C and have a characteristic outer membrane
41 or ‘toga’. Additionally, 16S rRNA sequences of this group have been isolated at lower
42 temperatures suggesting that the temperature growth range of this order is much greater (Nesbo,
43 *et al.*, 2006, Nesbo, *et al.*, 2010). Not surprisingly therefore, a member of this ‘mesotoga’ group
44 has been grown from an anaerobic reactor, and grows best at 40°C but not above 50°C (Ben
45 Hania, *et al.*, 2011, Nesbo, *et al.*, 2012). However, most isolated thermophilic members have
46 been obtained from deep-sea and terrestrial hydrothermal systems, oil reservoirs and some from
47 thermophilic anaerobic reactors and include genera such as *Thermotoga*, *Thermosipho*,
48 *Mesotoga*, *Fervidobacterium*, *Geotoga*, *Petrotoga*, *Marinitoga*, *Kosmotoga*, *Oceanotoga*, and
49 *Defluviitoga* (Ben Hania, *et al.*, Andrews & Patel, 1996, Antoine, *et al.*, 1997, Wery, *et al.*, 2001,
50 L’Haridon, *et al.*, 2002, DiPippo, *et al.*, 2009, Ben Hania, *et al.*, 2011, Jayasinghearachchi & Lal,
51 2011, Nesbo, *et al.*, 2012). The first isolates of the *Thermotogales* were from marine hot spring
52 environments and most were extreme thermophiles. Some of the moderate thermophilic
53 *Thermotogales* isolated from deep-sea vents are *Marintoga* (Wery, *et al.*, 2001, Alain, *et al.*,
54 2002, Postec, *et al.*, 2005, Nunoura, *et al.*, 2007, Postec, *et al.*, 2010) and *Thermosipho* (e.g
55 (Takai & Horikoshi, 2000, Urios, *et al.*, 2004). Although there is a range of optimum growth
56 temperatures that members of the *Thermotogales* grow at, all grow at near neutral pH (6.5-7.0).
57 Here we describe the first moderately acidophilic *Thermotogales* that forms a distinct
58 phylogenetic lineage within the *Thermotogales*.

59

60 Strain cd-1655R^T was isolated from a hydrothermal vent deposit (“chimney”) from the Mariner
61 vent field (176 54.17’W, 22 16.25’S, depth of 1925 m, sample number J2-448-9-R1) along the

62 Eastern Lau Spreading Center and Valu Fa Ridge in the Southwestern Pacific. Deep-sea
63 hydrothermal vent deposits (“chimneys”) were collected in July 2009 using *ROV Jason II*. The
64 pH of the high temperature (>300°C) hydrothermal fluids being emitted from these deposits was
65 about pH 2.8, but the pH in the deposits could not be measured. Individual samples were placed
66 in specially designed insulated containers and brought to the surface. Once shipboard, samples
67 were quickly processed as described previously (Gotz, *et al.*, 2002, Reysenbach, *et al.*, 2006),
68 and stored anaerobically at 4°C.

69

70 Samples of the hydrothermal deposit slurry were inoculated in the medium as described by
71 Reysenbach *et al.*, 2006. Because relatives of the *Thermoplasmatales* were detected in clone
72 libraries from samples from this same site in 2005 (Reysenbach, *et al.*, 2006), enrichments were
73 incubated at 60°C and monitored for changes in turbidity. After two days, the enrichments were
74 examined under phase microscopy and were primarily rods with an outer sheath-like structure
75 (“toga”). Cultures were subsequently purified by several series of dilution-to-extinction transfers
76 and their purity verified by 16S rRNA gene sequencing. Strain cd-1655R^T was chosen for further
77 characterization. Subsequent growth studies were done in triplicate at pH 5.5, 60°C and direct
78 cell counts were done using a Petroff-Hauser counting chamber.

79

80 The morphology of strain cd-1655R^T was further examined using transmission electron
81 microscopy as described previously (Flores, *et al.*, 2011). The cells were coccoid to rod-shaped,
82 occurring singly or in pairs with a diameter of about 0.4 µm in diameter and about 0.8-1.0 µm
83 long (Fig 1). Cells were surrounded by the typical *Thermotogales* sheath-like outer structure or

84 'toga'. In some cases dividing cells were surrounded by a single sheath. Cells were Gram-
85 negative and no spores were observed. Cells were motile, with peritrichous flagella.

86

87 Carbon sources were tested at 0.1% (w/v, v/v) with and without CO₂ in the headspace (N₂,
88 100%), with sulfur as the sole electron acceptor, in the presence of 0.02% yeast extract. All
89 cultures were transferred at least once to ensure there was no substrate carry over. Substrates
90 tested included yeast extract, peptone, maltose, sucrose, xylose, starch, ribose, tryptone, glucose,
91 casamino acids, pyruvate and glycerol. Strain cd-1655R^T grew on yeast extract, peptone,
92 maltose, sucrose, glucose, xylose, ribose, starch, tryptone and grew poorly on fructose. Sulfite (5
93 mM), nitrate (20 mM), cystine (0.05% w/v), and nitrite (5 mM) could not be used as electron
94 acceptors. Although elemental sulfur (~1% w/v) could be used as the sole electron acceptor,
95 optimal growth was achieved in the presence of cystine. Growth was not stimulated with
96 thiosulfate (20 mM) as the electron acceptor, although poor growth did occur with thiosulfate as
97 the sole electron acceptor.

98

99 Strain cd-1655R^T grew between 45 and 65°C, growing best between 57-60°C (Table 1) in a
100 medium with 0.2% yeast extract and sulfur as the electron acceptor. No growth was detected at
101 40 and 70°C. The isolate grew in media at pH 4.1-6.0, and could not grow at pH 3.7 or pH 6.5,
102 growing optimally at pH 5.5-5.7. Under optimal conditions, the doubling time of strain cd-
103 1655R^T was about 180 min. No growth occurred at 0.5% or 6.0% NaCl, and optimal growth was
104 at 3% (w/v) NaCl. In media reduced with cystine, poor growth occurred in 0.75% O₂, but no
105 growth occurred at 1.5% O₂ or higher.

106

107 Genomic DNA was extracted from isolated cultures using the DNeasy Tissue Kit (Qiagen)
108 following the manufacturer's protocol. For the DNA base composition, DNA was extracted
109 according to Wilson (1997) and the cesium chloride gradient purification was omitted. The DNA
110 base composition was determined by thermal denaturation (Marmur & Doty, 1962) and was
111 about 45 G+C mol%. Analysis of fatty acids was done as previously described (Flores, *et al.*,
112 2011). Besides regular C₁₂-C₂₀ fatty acids, strain cd-1655R^T had small amounts of 15,16-
113 dimethyltriacontanedioic acid ('diaboloic acid') and 15,16-dimethyl-30-glyceryloxytriacontanoic
114 acid (Table 2), diagnostic for *Thermotogales* (Damste, *et al.*, 2007). The polar lipids mainly
115 consisted of ornithine lipids and phospholipids with a phosphoethanolamine head group.
116
117 The 16S rRNA gene from the isolate was amplified, purified and sequenced as described
118 previously (Reysenbach, *et al.*, 2006). Nearly complete 16S rRNA gene sequence was assembled
119 in SeqMan and compared to the NCBI non-redundant database using BLAST (Altschul *et al.*,
120 1997). The strain cd-1665R^T 16S rRNA sequence was over 98% similar to the cloned 16S rRNA
121 gene sequences from hydrothermal deposits samples from the Kermadec arc (Stott, *et al.*, 2008)
122 and the Southern Mariana vent fields (Kato, *et al.*, 2010). Furthermore, the isolate was related
123 (~93% 16S rRNA sequence similarity) to sequences obtained from hydrothermal samples in the
124 Okinawa Trough (Inagaki, *et al.*, 2006) and Yellowstone National Park (unpublished).
125 Additionally, sequences similar to that of cd-1665R^T were detected in a large pyro-tagged 16S
126 rRNA gene database from deep-sea vents from the Mid-Atlantic Ridge (Flores, *et al.*, 2011) and
127 Mariner vents along the ELSC (Flores *et al.*, 2012) but not from Guaymas Basin vent deposits.
128 EZtaxon (Chun, *et al.*, 2007) placed strain cd-1665R^T within the *Thermotogales* and *Firmicutes*
129 with its 16S rRNA gene sequence being 82.72% similar to *Thermoanaerobacter thermocopriae*,

130 and 82.17% to *Kosmotoga arenicorallina*. However, using manual alignments in ARB (Ludwig,
131 *et al.*, 2004) and based on secondary structure constraints, strain cd-1655R^T was most closely
132 related to members of the genus *Kosmotoga* (still only ~82% sequence similarity).

133
134 Initial phylogenetic analysis was done as described in Flores *et al.*, 2011 using both ARB and
135 MEGA5 (Tamura *et al.*, 2011). Using maximum likelihood analysis (MEGA and RAxML)
136 (Stamatakis *et al.*, 2008), and a balanced inclusion of most of the major lineages within the
137 Bacteria, strain cd-1655R^T invariably formed a new deeply branching member of the
138 Thermotogales, with strong bootstrap support (100%, Fig 2), separate from the *Dictyoglomi*
139 (Zhaxybayeva, *et al.*, 2009, Nishida, *et al.*, 2011). The strain cd-1655R^T has been selected for
140 genome sequencing by the US Department of Energy-Joint Genome Institute, and obtaining
141 more insights into the genomic content of this new *Thermotogales* will help resolve its
142 phylogenetic position further. Furthermore, when the analysis was restricted to sequences from
143 the *Thermotogales* and *Firmicutes*, strain cd-1655R^T branches between the phyla. However,
144 given the strong bootstrap support of strain cd-1655R^T in multiple phylogenetic analyses, its
145 clear 'toga' and diagnostic *Thermotogales* fatty acids, strain cd-1655R^T is undoubtedly a member
146 of the *Thermotogales*.

147
148 Strain cd-1655R^T forms a distinct deeply diverging lineage within the *Thermotogales*, and is
149 closely related to sequences obtained from environmental surveys from other deep-sea and
150 terrestrial hot springs. However, its closest relative in culture (*Kosmotoga*) is only about 82%
151 similar in 16S rRNA sequence. Furthermore, this marine *Thermotogales*, is the first
152 *Thermotogales* that is a thermoacidophile, growing optimally at pH 5.5-5.7, but unable to grow

153 at pH 6.5. Like some of the moderate thermophilic *Thermotogales*, it can grow poorly in the
154 presence of low oxygen, uses sulfur and thiosulfate as an electron acceptor, has a 'toga' and is
155 motile. Based on comparative physiological and phylogenetic data, we propose that strain cd-
156 1655R^T is a new species of a novel genus in the *Thermotogales*, and propose the name
157 *Mesoaciditoga lauensis*.

158

159 **Description of *Mesoaciditoga* gen. nov.**

160 *Mesoaciditoga*: Me.so.a.ci.di.to'ga. Gr. adj. *mesos*, middle; N.L. n. acidum (from L.
161 adj. *acidus* -a -um, sour, tart, acid), an acid; L. fem. n. *toga*, Roman outer garment, toga; N.L.
162 fem. n. *Mesoaciditoga*, a moderate acidophilic toga.

163

164 Cells are short rods to cocci, with a sheath-like outer structure. Cells occur singly or in pairs, are
165 Gram negative and do not produce spores. Moderately thermoacidophilic, anaerobic
166 chemoorganotroph able to ferment a range of carbohydrates, proteinaceous substrates and yeast
167 extract. Reduces sulfur. The DNA G + C content of the type strain is 45 mol% (T_m). The 16S
168 rRNA gene sequence places the genus *Laodiceanella* in a deeply diverging lineage within the
169 *Thermotogales*. The type species is *Mesoaciditoga lauensis*.

170

171 **Description of *Mesoaciditoga lauensis* sp. nov.**

172 *Mesoaciditoga lauensis* (lau.en'sis. N.L. fem. adj. *lauensis* of deep-sea vents in the Lau basin in
173 the Southwestern Pacific.

174

175 In addition to the characteristics of the genus description, is a moderate thermoacidophilic non-
176 sporulating rod of about 0.4-0.5 to about 0.8-1.0 μm long, occurring singly or in pairs. Cells are

177 motile with multiple flagella. Growth occurs between 45 and 65°C (optimum 57-60°C), at pH of
178 4.1-6.0 (optimum pH 5.5-5.7) and NaCl concentrations between 1 and 5% (w/v) (optimum
179 3.0%). Doubling time is 180 min. Chemoorganotrophic, grows on yeast extract, peptone,
180 maltose, sucrose, fructose, glucose, tryptone, starch and xylose. Yeast extract and cystine
181 enhance growth. Reduces elemental sulfur to hydrogen sulfide. The 16S rRNA sequence
182 similarity to *Kosmotoga arenicorallina* is about 82%.

183

184 The type strain is cd-1655R^T (=DSMZ 25116, OCM 1212), was isolated from a deep-sea
185 hydrothermal vent deposit in the Mariner vent field in the Eastern Lau Spreading Center,
186 southwestern Pacific. The DNA G + C content is 45 mol %.

187

188 **Acknowledgments**

189 We thank Olga Zhaxybayeva and Camille Nesbø for very fruitful discussions and guidance in the
190 phylogenetic treatment of the data. Thanks to the crew and pilots of the R/V *Thomas G.*
191 *Thompson* and DSROV *Jason II* for collecting the samples, Ellen Hopmans and Irene Rijpstra
192 for assistance with polar lipid identifications and Dianne Moyles for the electron microscopy
193 work. This research was funded by the United States National Science Foundation grants OCE-
194 0752469 and OCE-0937404 to A.-L.R.

195

196

198 **References**

- 199 **Alain, K., Marteinsson, V.T., Miroshnichenko, M.L., Bonch-Osmolovskaya, E.A., Prieur,**
200 **D. & Birrien, J.L. (2002).** *Marinitoga piezophila* sp. nov., a rod-shaped, thermo-piezophilic
201 bacterium isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *Int J Syst*
202 *Evol Microbiol* **52**, 1331-1339.
- 203 **Alain, K., Marteinsson, V.T., Miroshnichenko, M.L., Bonch-Osmolovskaya, E.A., Prieur,**
204 **D. & Birrien, J.L. (2002).** *Marinitoga piezophila* sp. nov., a rod-shaped, thermo-piezophilic
205 bacterium isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. *Int J Syst*
206 *Evol Microbiol* **52**, 1331-1339.
- 207 **Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, Z., Miller, W. & Lipman, D.J.**
208 **(1997).** Gapped BLAST and PSI-BLAST: A new generation of protein database search
209 programs. *Nucl Acids res* **25**, 3389-3402.
- 210 **Andrews, K.T. & Patel, B.K. (1996).** *Fervidobacterium gondwanense* sp. nov., a new
211 thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the
212 Great Artesian Basin of Australia. *Int J Syst Bacteriol* **46**, 265-269.
- 213 **Antoine, E., Cilia, V., Meunier, J. R., Guezennec, J., Lesongeur, F. & Barbier, G. (1997).**
214 *Thermosipho melanesiensis* sp. nov., a new thermophilic anaerobic bacterium belonging to the
215 order Thermotogales, isolated from deep-sea hydrothermal vents in the southwestern Pacific
216 Ocean. *Int J Syst Bacteriol* **47**, 1118-1123.
- 217 **Ben Hania, W., Godbane, R., Postec, A., Hamdi, M., Ollivier, B. & Fardeau, M.-L. (2012).**
218 Isolation and characterization of *Defluviitoga tunisiensis* gen. nov, sp. nov., a novel thermophilic
219 bacterium pertaining to the order Thermotogales, isolated from a mesothermic anaerobic reactor
220 treating cheese whey in Tunisia. *Int J Syst Evol Microbiol*. **62**, 1377-1382.
- 221 **Ben Hania W., Ghodbane, R., Postec, A., Brochier-Armanet, C., Hamdi, M., Fardeau, M.-**
222 **L. & Ollivier, B. (2011).** Cultivation of the first mesophilic representative ("mesotoga") within
223 the order Thermotogales. *Syst Appl Microbiol* **34**, 581-585.
- 224 **Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y.-W. (2007).** EzTaxon:
225 a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene
226 sequences. *Int J Syst Evol Microbiol* **57**, 2259-2261.
- 227 **Damsté, J. S., Rijpstra, W.I., Hopmans, E. C., Schouten, S., Balk, M. & Stams, A. J. (2007).**
228 Structural characterization of diabolic acid-based tetraester, tetraether and mixed ether/ester,
229 membrane-spanning lipids of bacteria from the order Thermotogales. *Arch Microbiol* **188**, 629-
230 641.
- 231 **Davey, M. E., Wood, W. A., Key, R., Nakamura, K. & Stahi, D. (1993).** Isolation three
232 species of *Geotoga* and *Petrotoga*: Two new genera representing a new lineage in the bacterial
233 line of descent distantly related to the "Thermotogales". *Syst Appl Microbiol* **16**, 191-200.
- 234 **DiPippo, J. L., Nesbø, C. L., Dahle, H., Doolittle, W. F., Birkland, N.-K. & Noll, K. M.**
235 **(2009).** *Kosmotoga olearia* gen. nov., sp. nov., a thermophilic, anaerobic heterotroph isolated
236 from an oil production fluid. *Int J Syst Evol Microbiol* **59**, 2991-3000.
- 237 **Flores, G. E., Hunter, R. C., Liu, Y., Mets, A., Schouten, S. & Reysenbach, A.-L. (2012).**
238 *Hippea jasoniae* sp. nov. and *Hippea alviniae* sp. nov., thermoacidophilic Deltaproteobacteria
239 isolated from deep-sea hydrothermal vent deposits. *Int J Syst Evol Microbiol* **62**, 1252-1258.
- 240 **Flores, G. E., Campbell, J. H., Kirshtein, J. D., Meneghin, J., Podar, M., Steinberg, J. I.,**
241 **Seewald, J. S., Tivey, M. K., Voytek, M. A., & other authors (2011).** Microbial community

242 structure of hydrothermal deposits from geochemically different vent fields along the Mid-
243 Atlantic Ridge. *Environ Microbiol* **8**, 2158-2171.

244 **Flores, G.E., Shakya, M., Meneghin, J., Yang, Z.K., Seewald, J.S., Wheat, C.G., Podar, M.**
245 **and Reysenbach, A.-L. (2012).** Inter-field variability in the microbial communities of
246 hydrothermal vent deposits from a back-arc basin. *Geobiology* **10**: 333-346.

247 **Götz, D., Banta, A., Beveridge, T. J., Rushdi, A. I., Simoneit, B. R. & Reysenbach, A.-L.**
248 **(2002).** *Persephonella marina* gen. nov., sp. nov. and *Persephonella guaymasensis* sp. nov., two
249 novel, thermophilic, hydrogen-oxidizing microaerophiles from deep-sea hydrothermal vents. *Int*
250 *J Syst Evol Microbiol* **52**, 1349-1359.

251 **Huber, R., Langworthy, T. A., König, H., Thomm, M., Woese, C. R., Steytr, U. B. &**
252 **Stetter, K. O. (1986).** *Thermotoga maritima* sp. nov. represents a new genus of uniquely
253 extremely thermophilic eubacteria growing up to 90 °C. *Arch Microbiol* **144**, 324–333.

254 **Huber, R., Woese, C. R., Langworthy, T. A., Fricke, H. & Stetter, K. O. (1989).**
255 *Thermosipho africanus* gen. nov., represents a new genus of thermophilic eubacteria within the
256 “thermotogales”. *Syst Appl Microbiol* **12**, 32–37.

257 **Huber, R., Woese, C. R., Langworthy, T. A., Kristjansson, J. K. & Stetter, K. O. (1990).**
258 *Fervidobacterium islandicum* sp. nov., a new extremely thermophilic eubacterium belonging to
259 the “Thermotogales”. *Arch Microbiol* **154**, 105–111.

260 **Inagaki, F., Kuypers, M.M.M., Tsunogai, U., Ishibashi, J., Nakamura, K., Treude, T.,**
261 **Ohkubo, S., Nakaseama, M., Gena, K., Chiba, H. & other authors (2006).** Microbial
262 community in a sediment-hosted CO₂ lake of the southern Okinawa Trough hydrothermal
263 system. *Proc Natl Acad Sci U S A* **103**, 14164-14169.

264 **Jannasch, H. W., Huber, R., Belkin, S. & Stetter, K. O. (1988).** *Thermotoga neapolitana* sp.
265 nov. of the extremely thermophilic eubacterial genus *Thermotoga*. *Arch Microbiol* **150**, 103–104.

266 **Jayasinghearachchi, H. S. & Lal, B. (2011).** *Oceanotoga teriensis* gen. nov., sp. nov., a
267 thermophilic bacterium isolated from offshore oil-producing wells. *Int J Syst Evol Microbiol* **61**,
268 554-560.

269 **Kato, S., Takano, Y., Kakegawa, T., Oba, H., Inoue, K., Kobayashi, C., Utsumi, M.,**
270 **Marumo, K., Kobayashi, K. & other authors (2010).** Biogeography and Biodiversity in
271 Sulfide Structures of Active and Inactive Vents at Deep-Sea Hydrothermal Fields of the
272 Southern Mariana Trough. *Appl Environ Microbiol* **76**, 2968-2979.

273 **L'Haridon, S., Miroshnichenko, M. L., Hippe, H., Fardeau M.-L., Bonch-Osmolovskaya, E.**
274 **A., Stackebrandt, E. & Jeanthon, C. (2002).** *Petrotoga olearia* sp. nov. and *Petrotoga sibirica*
275 sp. nov., two thermophilic bacteria isolated from a continental petroleum reservoir in Western
276 Siberia. *Int J Syst Evol Microbiol* **52**, 1715-1722.

277 **Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhumkumar, Buchner, A.,**
278 **Lai, T., Steppi, S. & other authors (2004).** ARB: a software environment for sequence data.
279 *Nucleic Acids Res* **32**, 1363-1371.

280 **Marmur, J. & Doty, P. (1962).** Determination of the base composition of deoxyribonucleic acid
281 from its thermal denaturation temperature. *J Mol Biol* **5**, 109-118.

282 **Nesbø, C. L., Dlutek, M., Zhaxybayeva, O. & Doolittle, W. F. (2006).** Evidence for existence
283 of "mesotogas," members of the order Thermotogales adapted to low-temperature environments.
284 *Appl Environ Microbiol* **72**, 5061-5068.

285 **Nesbø, C. L., Kumaraswamy, R., Dlutek, M., Doolittle, W. F. & Foght, J. (2010).** Searching
286 for mesophilic Thermotogales bacteria: "mesotogas" in the wild. *Appl Environ Microbiol* **76**,
287 4896-4900.

288 **Nesbø C. L., Bradnan D. M., Adebunsi A., Dlutek, M., Petrus, A. K., Foght, J., Doolittle,**
289 **W. F., Noll, K. M. (2012).** *Mesotoga prima* gen. nov., sp. nov., the first described mesophilic
290 species of the Thermotogales. *Extremophiles* **16**: 387-393.

291 **Nishida, H., Beppu, T. & Ueda, K. (2011).** Whole-genome comparison clarifies close
292 phylogenetic relationships between the phyla Dictyoglomi and Thermotogae. *Genomics* **98**, 370-
293 375.

294 **Nunoura, T., Oida, H., Miyazaki, M., Suzuki, Y., Takai, K. & Horikoshi, K. (2007).**
295 *Marinitoga okinawensis* sp. nov., a novel thermophilic and anaerobic heterotroph isolated from a
296 deep-sea hydrothermal field, Southern Okinawa Trough. *Int J Syst Evol Microbiol* **57**, 467-471.

297 **Postec, A., Ciobanu, M., Birrien, J.-L., Bienvenu, N., Prieur, D. & Le Romancer, M. (2010).**
298 *Marinitoga litoralis* sp. nov., a thermophilic, heterotrophic bacterium isolated from a coastal
299 thermal spring on Ile Saint-Paul, Southern Indian Ocean. *Int J Syst Evol Microbiol* **60**, 1778-
300 1782.

301 **Postec, A., Le Breton, C., Fardeau, M.-L., Lesongeur, F., Pignet, P., Querellou, J., Ollivier,**
302 **B. & Godfroy, A. (2005).** *Marinitoga hydrogenitolerans* sp. nov., a novel member of the order
303 *Thermotogales* isolated from a black smoker chimney on the Mid-Atlantic Ridge. *Int J Syst Evol*
304 *Microbiol* **55**, 1217-1221.

305 **Ravot, G., Ollivier, B., Magot, M., Patel, B., Crolet, J., Fardeau, M. & Garcia, J. (1995).**
306 Thiosulfate reduction, an important physiological feature shared by members of the order
307 thermotogales. *Appl Environ Microbiol* **61**, 2053-2055.

308 **Reysenbach, A.-L., Liu, Y., Banta, A. B., Beveridge, T. J., Kirshtein, J. D., Schouten, S.,**
309 **Tivey, M. K., Von Damm, K. L. & Voytek, M. A. (2006).** A ubiquitous thermoacidophilic
310 archaeon from deep-sea hydrothermal vents. *Nature* **442**, 444-447.

311 **Stamatakis, A., Hoover, P. & Rougemont, J. (2008).** A rapid bootstrap algorithm for the
312 RAxML web servers. *Syst Biol* **57**, 758-771.

313 **Stott, M. B., Saito, J. A., Crowe, M. A., Dunfield, P. F., Hou, S., Nakasone, E., Daughney,**
314 **C. J., Smirnova, A. V., Mountain, B. W. & other authors (2008).** Culture-independent
315 characterization of a novel microbial community at a hydrothermal vent at Brothers volcano,
316 Kermadec arc, New Zealand. *J. Geophys. Res.* **113**, B08S06.

317 **Takai, K. & Horikoshi, K. (2000).** *Thermosipho japonicus* sp. nov., an extremely thermophilic
318 bacterium isolated from a deep-sea hydrothermal vent in Japan. *Extremophiles* **4**, 9-17.

319 **Tamura, K., Peterson, D., Peterson, N., Stecher G., Nei M., and Kumar, S. (2011).** MEGA5:
320 Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and
321 maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731-2739.

322 **Urios, L., Cuff-Gauchard, V., Pignet, P., Postec, A., Fardeau, M.-L., Ollivier, B. &**
323 **Barbier, G. (2004).** *Thermosipho atlanticus* sp. nov., a novel member of the Thermotogales
324 isolated from a Mid-Atlantic Ridge hydrothermal vent. *Int J Syst Evol Microbiol* **54**, 1953-1957.

325 **Wery, N., Lesongeur, F., Pignet, P., Derennes, V., Cambon-Bonavita, M. A., Godfroy, A. &**
326 **Barbier, G. (2001).** *Marinitoga camini* gen. nov., sp. nov., a rod-shaped bacterium belonging to
327 the order Thermotogales, isolated from a deep-sea hydrothermal vent. *Int J Syst Evol Microbiol*
328 **51**, 495-504.

329 **Wilson, K. (1997).** Preparation of genomic DNA from bacteria. In *Current Protocols in*
330 *Molecular Biology*, pp. 2.4.1–2.4.5. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D.
331 Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York, NY: Greene Publishing and Wiley-
332 Interscience.

333 **Zhaxybayeva, O., Swithers, K. S., Lapierre, P., Fournier, G. P., Bickhart, D. M., DeBoy, R.**
334 **T., Nelson, K.E., Nesbø, C. L., Doolittle, W. F. & other authors (2009).** On the chimeric
335 nature, thermophilic origin, and phylogenetic placement of the Thermotogales. *Proc Natl Acad*
336 *Sci U S A* **106**, 5865-5870.

Table 1. Characteristics that distinguish strain cd-1655R^T from other marine hydrothermal *Thermotogales* genera.

Taxa: 1, cd-1655R^T (data from this study); 2, *Marinitoga camini* DSM 13578^T (Wery *et al.*, 2001); 3, *Thermotoga maritima* DSM 3109^T (Huber, *et al.*, 1986, Ravot, *et al.*, 1995); 4, *Thermosipho japonicus* IHB1^T (Takai & Horikoshi, 2000); 5, *Kosmotoga arenicorallina* DSM 22549^T (Nunoura *et al.*, 2010); 6, *Mesotoga prima* DSM 24739^T (Nesbø *et al.*, 2012), 7, *Oceanotoga teriensis* OCT74^T (Jayasinghearachchi & Lal, 2011).

Characteristic	1	2	3	4	5	6	7
Temperature range °C (opt)	45-65 (60)	25-65 (55)	55-90 (80)	45-80 (65)	50-65 (60)	20-50 (37)	25-70 (55-58)
pH range (opt)	4.1-6.0 (5.7)	5-9 (7)	5.5-9.0 (6.5)	5.0-9.0 (6.0)	6.2-8.0 (7.1)	6.5-8.0 (7.5)	5.5-9.0 (7.3-7.8)
NaCl range % (opt)	1-5 (3)	1.0-4.5 (2)	0.25-6.0 (2.7)	2.0-6.0 (3.0)	1.0-6.0 (3.0)	2.0-6.0 (4.0)	0-12 (4.0-4.5)
Doubling time (min)	180	102	75	72	150	990	60-90
DNA G + C (mol%)	45	29	46	33	40.8	45.3	26.8
Flagella	+	+	+	-		-	+
Electron acceptor	S ⁰ , S ₂ O ₃ ^{2-±} cystine enhances growth	S ⁰ , cystine	S ⁰ , S ₂ O ₃ ²⁻	S ⁰ , S ₂ O ₃ ²⁻	S ⁰ , cystine	S ⁰ , S ₂ O ₃ ²⁻ , SO ₃ ²⁻	S ⁰ , S ₂ O ₃ ²⁻
Substrate utilization							
Glucose	+	+	+	+	ND	±	+
Maltose	+	+	+	+	+	+	-
Ribose	+	-	+	ND	-	+	+
Fructose	±	+	+	ND	-	+	+
Sucrose	+	+	+	+	-	+	+
Xylose	-	-	+	-	+	+	+
Glycerol	-	-	-	ND	+	-	ND
Pyruvate	-	+	-	ND	-	±	ND
Tryptone	+	+	ND	ND	-	+	+
Starch	+	+	+	+	-	ND	+
Casamino acids	-	-	-	-	-	+	ND
Yeast Extract	+	+	+	+	+	+	+

± weakly supported or enhanced growth

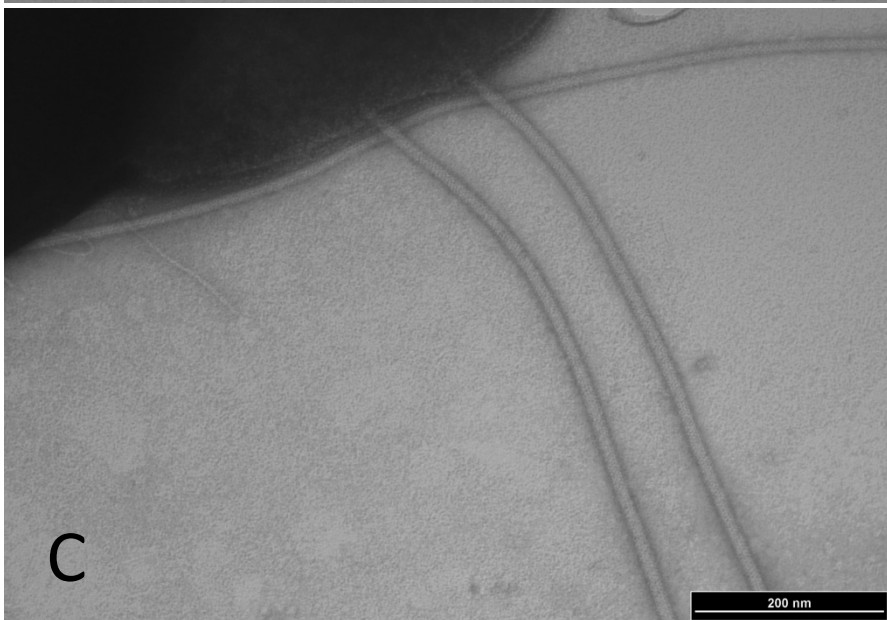
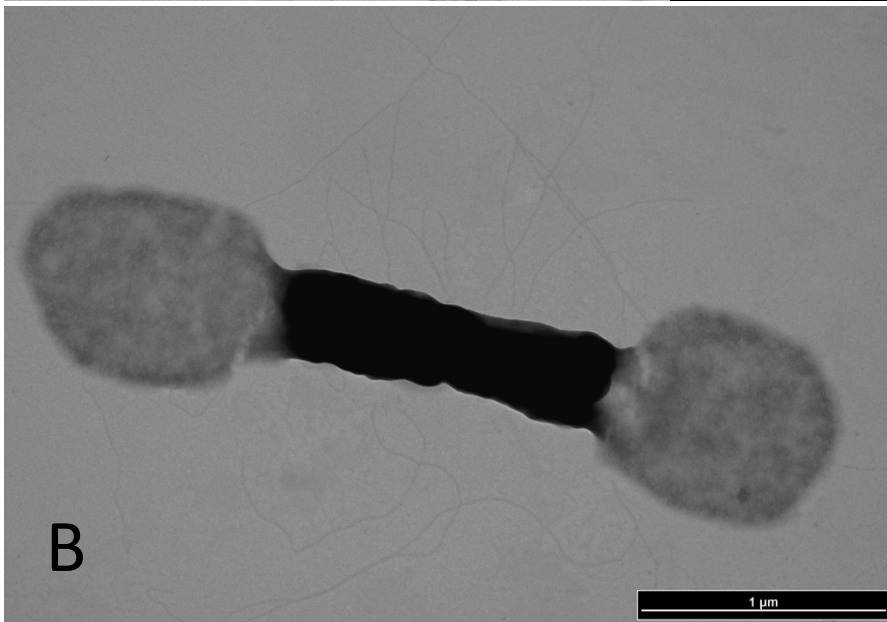
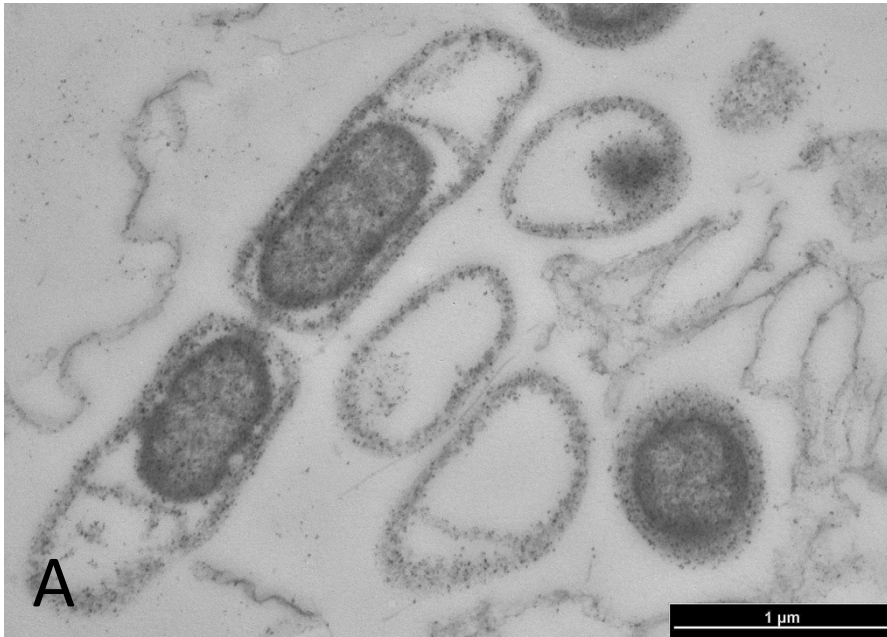
Table 2. Fatty acid lipid composition (%) of strain cd-1655R^T

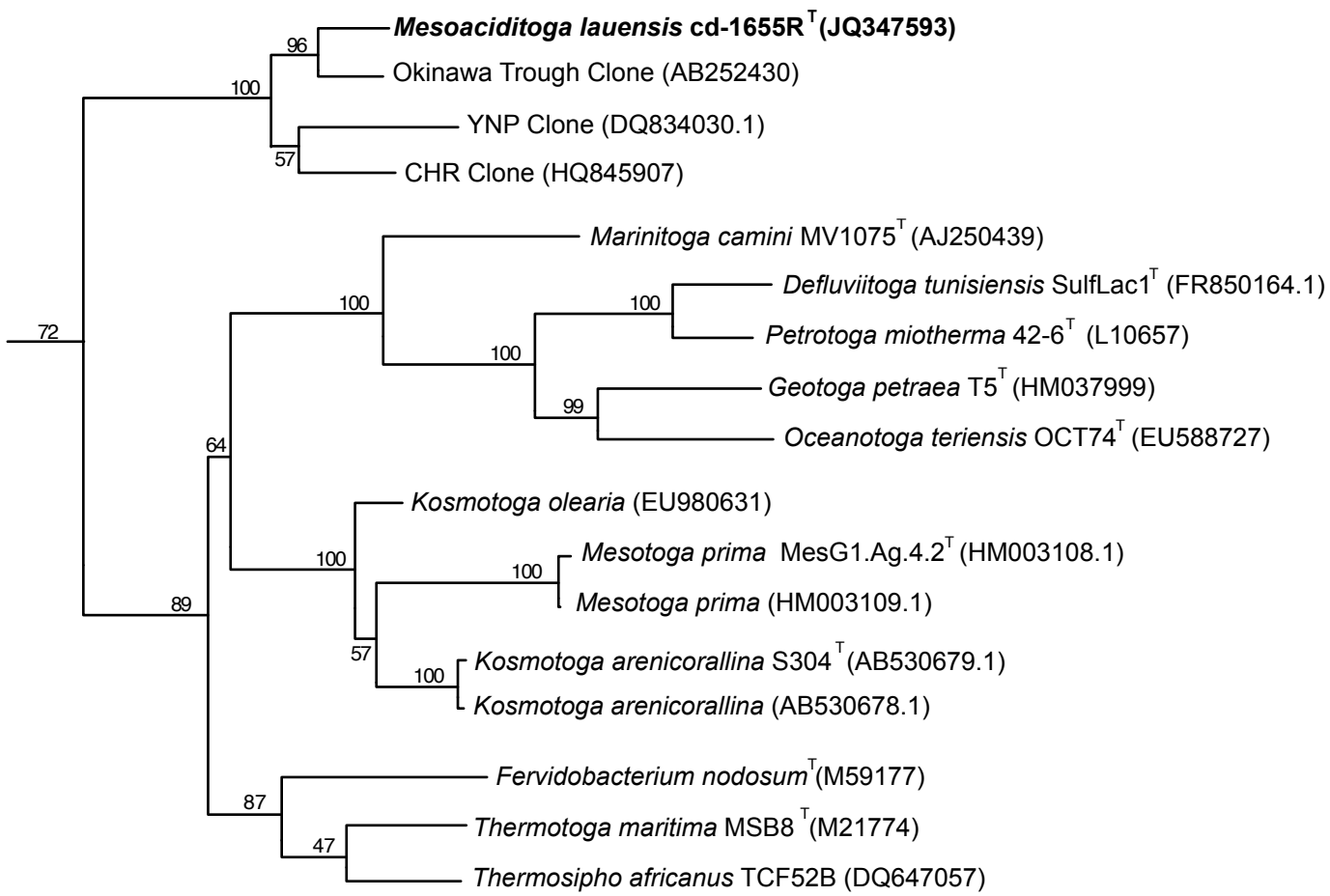
C12:0	1.0
C14:1 ω 9	0.7
C14:0	7.5
C16:1 ω 9	10.2
C16:0	65.7
C16:0 10 methyl	0.9
C17:0 iso	0.4
C18:1 ω9	2.3
C18:1 ω7	0.9
C18:0	3.1
C20:1 ω9	4.0
C20:1 ω7	0.5
C22:1 ω9	0.8
C30 diFA 15,16 dimethyl	1.9
C33 15,16-dimethyl-30-glyceryloxytriacontanoic acid	0.2

Figure legends

Fig 1. A. Thin section TEM image of strain cd-1655^T showing the sheath-like membrane or 'toga'. B. TEM of negatively stained rods within a 'toga' and with multiple flagella. C. High magnification TEM of negatively stained cells showing details of flagella.

Fig 2. Maximum-likelihood topology based on 16S rRNA gene sequences, showing the position of strain cd-1655R^T relative to members of the *Thermotogales* and selected taxa within the *Dictyoglomi* and *Firmicutes*. The optimal maximum likelihood tree obtained for the dataset was constructed using the GTR+GAMMA model as implemented in RAxML v.7.2.8 (Stamatakis et al., 2008). Support values for nodes were generated via a 500 bootstrap replicate search as implemented in RAxML. The scale bar represents 0.2 changes per nucleotide position. The following fourteen 16S rRNA gene sequences were used as outgroup taxa to construct the phylogeny but are not shown in topology: *Alkaliphilus transvaalensis* SAGM1^T (AB037677), *Aquifex pyrophilus* Kol5a^T (M83548), *Bacillus subtilis* W168 (K00637), *Clostridium botulinum* (CP000727), *Clostridium thermocopriae* IAM 13577 (L09167.1), *Dictyoglomus thermophilum* H-6-12 (X69194.1), *Dictyoglomus turgidum* DSM 6274 (CP001251.1), *Escherichia coli* (J01695), *Flexibacter flexilis* ATCC 23079^T (M62794), *Marinithermus hydrothermalis* T1^T(AB079382), *Methanocaldococcus jannaschii* JAL-1^T (L77117), *Persephonella marina* EX-H1^T (AF188332), *Thermus thermophilus* HB8^T (X07998), and uncultured bacterium clone LHC3_L4_B12 (EU924243.1).





0.2