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1 ***Alkaliphilus serpentinus* sp. nov. and *Alkaliphilus pronyensis* sp. nov., two novel anaerobic**
2 **alkaliphilic species isolated from the serpentinite-hosted Prony Bay Hydrothermal Field**
3 **(New Caledonia).**

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13

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15 Subject category: *Firmicutes* (Class *Clostridia*, Order *Clostridiales*)

16

17 The GenBank accession numbers for the whole genome sequence and the 16S rRNA gene
18 sequence of strain LacT^T are respectively WBZB000000000 and KJ159208.

19 The GenBank accession numbers for the whole genome sequence and the 16S rRNA gene
20 sequence of strain LacV^T are respectively WBZC000000000 and KJ159207.

21

22 Keywords: *Firmicutes*, *Alkaliphilus*, anaerobic, hydrothermal vents, serpentization, New
23 Caledonia

24

25 **Abstract**

26 Two novel anaerobic alkaliphilic strains, designated as LacT^T and LacV^T, were isolated from
27 the Prony Bay Hydrothermal Field (PBHF, New Caledonia). Cells were motile, Gram-positive,
28 terminal endospore-forming rods, displaying a straight to curved morphology during the
29 exponential phase. Strains LacT^T and LacV^T were mesophilic (optimum 30°C), moderately
30 alkaliphilic (optimum pH 8.2 and 8.7, respectively) and halotolerant (optimum 2 % and 2.5 %
31 NaCl, respectively). Both strains were able to ferment yeast extract, peptone and casamino
32 acids, but only strain LacT^T could use sugars (glucose, maltose and sucrose). Both strains
33 disproportionated crotonate into acetate and butyrate. Phylogenetic analysis revealed that
34 strains LacT^T and LacV^T shared 96.4 % 16S rRNA gene sequence identity and were most
35 closely related to *A. peptidifermentans* Z-7036, *A. namsaraevii* X-07-2 and *A. hydrothermalis*
36 FatMR1 (95.7%–96.3%). Their genome size was of 3.29 Mb for strain LacT^T and 3.06 Mb for
37 strain LacV^T with a G+C content of 36.0 and 33.9 mol%, respectively. The ANI value between
38 both strains was 73.2%. Finally, strains LacT^T (= DSM 100337 = JCM 30643) and LacV^T (=
39 DSM 100017 = JCM 30644) are proposed as two novel species of the genus *Alkaliphilus*, order
40 *Clostridiales*, phylum *Firmicutes*, *Alkaliphilus serpentinus* sp. nov. and *Alkaliphilus pronyensis*
41 sp. nov., respectively. The genomes of the three *Alkaliphilus* species isolated from PBHF were
42 consistently detected in the PBHF chimney metagenomes, although at very low abundance, but
43 not significantly in the metagenomes of other serpentizing systems (marine or terrestrial)
44 worldwide, suggesting they represent indigenous members of the PBHF microbial ecosystem.

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49 **Introduction**

50 Serpentinization is a widespread geochemical process involving aqueous alteration of the
51 mantle rock peridotite to serpentinite and reduction of inorganic CO₂, resulting in an abundance
52 of potential energy sources, in the form of H₂, CH₄ and small organic molecules, that can be
53 used by microbial life [1]. However, these reactions also generate challenging conditions: high
54 pH, low availability of potential electron acceptors, and a low concentration of dissolved
55 inorganic carbon source.

56 Over the last two decades, serpentinization-driven microbial ecosystems have been the object
57 of increasing scientific interest, not only for the extremophilic nature of the microorganisms
58 thriving in such harsh conditions, but also because these environments represent the best
59 modern analogs of primitive hydrothermal systems existing at the Hadean where early proto-
60 cellular life may have arisen [2]. Recent next-generation sequencing studies, targeting 16S
61 rRNA gene, or true metagenomic analyses, have shown that all serpentinite-hosted ecosystems
62 share a low microbial diversity, largely dominated by few abundant bacterial phyla/classes
63 among which the *Betaproteobacteria*, the *Chloroflexi* and the *Clostridia*. The later one is
64 thought to be associated with the anaerobic micro-habitats of serpentinizing sites, where highly
65 reduced alkaline fluids are less mixed with oxygenated groundwater or seawater. *Clostridia*
66 dominated in various terrestrial alkaline seeps, such as The Cedars (California) [3], Manleluag
67 (Philippines) [4], Tablelands (Canada) [5], the deep alkaline groundwater of Cabeço de Vide
68 Aquifer (CVA, Portugal) [6], and the anoxic deep borehole water of Outokumpu (Finland) [7].
69 They were also dominant at the oceanic site of Lost City Hydrothermal Field (LCHF) near the
70 Mid Atlantic Ridge [8, 9] and in the marine shallow Prony Bay Hydrothermal Field (PBHF) in
71 New Caledonia [10, 11].

72 Metagenomic studies of serpentinite-hosted systems have revealed a high diversity and
73 abundance of [FeFe]-hydrogenases assigned to the *Firmicutes* orders *Thermoanaerobacterales*
74 and *Clostridiales* [5, 12]. The [FeFe]-hydrogenases are typically linked to H₂ production by
75 fermentations, or intra-cellular regulation of the reducing equivalents [13]. These enzymes thus
76 play essential functions in the anaerobic challenging environments of the serpentinitizing
77 systems.

78 Besides these ‘omics’ studies, a few cultivation-based approaches have been published. The
79 earliest one, conducted on alkaline springs of the Oman ophiolite, reported the cultivation of
80 aerobic heterotrophs belonging to *Firmicutes* [14], as did later similar studies on CVA
81 (Portugal) and Yanartaş (Turkey) terrestrials alkaline springs [15, 16]. Only one new species
82 was described, *Bacillus foraminis*, an aerobic, mesophilic, neutrophilic heterotroph, isolated
83 from the alkaline ground water of CVA [17]. Its phenotypic characteristics, however, were not
84 representative of typical serpentinite-hosted environment, and its belonging to the widespread
85 genus *Bacillus* questions its indigeneity. That prompts us to look for better model organisms
86 for physiological studies of adaptation to serpentinite-hosted environmental conditions by
87 tempting to isolate novel anaerobic, alkaliphilic bacteria from our predilected site of study, the
88 Prony Bay Hydrothermal Field in New Caledonia (PBHF). One target was the *Clostridiales*
89 since they represented almost a third of the bacterial community in the submarine chimneys of
90 PBHF [18]. The dominant phylotypes clustered with the *Clostridiales* from CVA (Portugal) [6]
91 and The Cedars (California) [3]. A phylotype related to *Dethiobacter alkaliphilus*, dominant in
92 juvenile chimneys of PBHF, was also proposed to be involved in the early organomineralization
93 of carbonates during the edification of the chimneys [19]. Our cultivation efforts resulted in the
94 isolation of several anaerobic strains belonging to *Clostridia*. They were described as novel
95 species or genus: *Vallitalea pronyensis* [20], *Acetoanaerobium pronyense* [21], *Clostridium* sp.

96 PROH2 [22], *Serpentinicella alkaliphila* [23] and *Alkaliphilus hydrothermalis* [24]. The later
97 was the first species described from a serpentinite-hosted system. Here we report the description
98 of strains LacT^T and LacV^T isolated from PBHF representing two novel species of the genus
99 *Alkaliphilus* (*Clostridiales*). We also investigated the distribution of the three PBHF
100 *Alkaliphilus* species genomes in metagenomes of various serpentinite-hosted environments
101 worldwide and discuss the potential role of the *Alkaliphilus* spp. in the PBHF ecosystem.

102

103 **Material and methods**

104 Sample collection

105 The Prony Bay Hydrothermal Field (PBHF) located in the south of New Caledonia is a
106 serpentinizing marine hydrothermal system composed of brucite-carbonate chimneys
107 discharging highly reduced alkaline fluids (up to pH 11.5) of low salinity enriched in hydrogen
108 and methane in a shallow lagoon. In the east of the central bay, active chimneys were sampled
109 during the 2011 HYDROPRONY campaign from site ST08 (22°21.598'S, 166°52.838'E) at 47
110 meters below sea level (mbsl) and site ST09 (22°21.653'S, 166°52.777'E) at 45 mbsl (Fig. S1)
111 [10, 11, 25]. Aboard the RV *Alis*, internal chimney parts were aseptically subsampled to avoid
112 contamination by the outer parts exposed to the surrounding seawater. Subsamples were further
113 ground aseptically with a pestle in a mortar under a continuous flux of N₂ gas to preserve
114 anaerobiosis and then distributed into sterile glass vials which were tightly closed with butyl
115 rubber stoppers and crimped with aluminum seals. The head space in the vials was slightly
116 pressurized (50 kPa) with 100% N₂ to prevent oxygen entrance. These samples were stored at
117 4°C until use.

118

119 Enrichment, isolation and cultivation

120 For the enrichment cultures, about 0.5 g of chimney slurry (ST08 or ST09) was used to inoculate
121 5 mL of basal medium (noted BM) which contained (per litre of distilled water): 2 g NaCl, 0.1
122 g KH_2PO_4 , 0.1 g K_2HPO_4 , 1 g NH_4Cl , 0.1 g KCl, 0.1 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.1 g CaCl_2 , 0.1 g Cystein-
123 HCl, 3 g Tris Base, 3 g $\text{Na}_2\text{S}_2\text{O}_3$, 1 g yeast extract and 1 mL Widdel's elements. The pH was
124 adjusted to 9.5 with 1M NaOH solution. The medium was boiled under a stream of O_2 -free N_2
125 gas and cooled to room temperature. Aliquots of 5 mL were dispensed into Hungate tubes,
126 degassed under $\text{N}_2:\text{CO}_2$ (80:20, v:v) and subsequently sterilized by autoclaving. Before
127 inoculation, 0.1 mL of 1M Lactate, 0.3 mL of 8 % (w/v) Na_2CO_3 and 0.1 mL of 2 % (w/v)
128 $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ were injected from sterile stock solutions into the tubes. The Hungate technique
129 was used throughout this study [26]. Isolation of colonies was performed three times
130 consecutively using the roll-tube method and 1.6 % (w/v) agar containing BM [26].

131

132 Microscopic analyses

133 Cells were routinely observed under a Nikon ECLIPSE E600 phase-contrast microscope for
134 culture monitoring. Cell structures and morphological characterization of strains LacT^{T} and
135 LacV^{T} deposited on Cu-grids were performed using a FEI Tecnai G2 transmission electron
136 microscope (TEM) operating at 200 kV. Exponentially grown cells were negatively stained
137 during 15 min with 2 % (w/v) sodium phosphotungstate (pH 7.0) for observation of flagella.
138 For observation of the cell wall, cells were first fixed 2 min with 10% (v/v) glutaraldehyde,
139 stained overnight with 2% (w/v) osmium tetraoxyde, and then embedded in EMbed-812 resin.
140 Embedded specimens were sliced into ultrathin sections (90 nm in thickness), placed on Cu
141 TEM grid and stained during 3 min with 2% (w/v) uranyl acetate and 1 min with 1% (w/v) lead
142 before observation.

143

144 Physiological tests

145 The pH, temperature, and NaCl concentration ranges for growth were determined in Hungate
146 tubes using basal medium (BM). Growth was determined by directly measuring the optical
147 density at 600 nm in Hungate tubes using a spectrophotometer equipped with a tube holder
148 (Cary 50, Varian). The BM pH was adjusted from 7.0 to 11.5 with anaerobic sterile stock
149 solutions of 1M HCl (acidic pH values), 10 % (w/v) NaHCO₃ or 8 % (w/v) Na₂CO₃ (basic pH
150 values) and was verified after inoculation. Tris Base in the medium was replaced by HEPES
151 (10 mM) for the low pH (7.0–8.0) values and by AMPSO (10 mM) for the high pH (10.0–11.5)
152 values. Culture experiments to determine pH range and optimum growth conditions were
153 performed at 30°C and 1 % NaCl. Temperature tests were performed at pH 9.0 and 1 % NaCl
154 using water baths from 15 °C to 55 °C. For NaCl requirement, NaCl was weighted directly in
155 the tubes prior to adding the medium (0 to 12 %) and cultures were performed at pH 9.0 and 30
156 °C. All cultures were performed in duplicate and reproduced twice. Growth rates were
157 determined for subcultures under the same experimental conditions.

158 Substrate utilization experiments were performed for each strain under its optimal growth
159 conditions (pH, temperature and NaCl) in a modified BM (yeast extract reduced to 0.5 g/L).
160 Sugars, organic acids and alcohol were tested each individually at 20 mM and proteinaceous
161 compounds at 5 g/L. Autotrophic growth was tested by replacing N₂ headspace by a H₂:CO₂
162 (80:20) at 200 kPa. Each compound was tested in duplicate Hungate tubes and those for which
163 positive growth was observed (OD significantly above that obtained on the inoculated duplicate
164 controls on modified BM only) were checked by two successive subcultures under the same
165 conditions. Bacterial growth was monitored by measuring the increase in turbidity at 600 nm
166 as mentioned above. H₂S production was determined measuring the formation of colloidal CuS
167 photometrically at 480 nm following the method described by Cord-Ruwisch [27]. Metabolic

168 end-products were measured by HPLC using an Aminex HPX-87H (Bio-Rad) column with
169 2.5 mM H₂SO₄ as mobile phase and gas-phase chromatography after 2-weeks of incubation at
170 30 °C. Fe(II) was measured as previously described [28].

171

172 Fatty acid and G+C content analyses

173 The determination of cellular fatty acids composition was performed at the DSMZ identification
174 service (Braunschweig, Germany). Cells were harvested in the late exponential growth phase
175 (c.a. $\sim 10^8$ cells. ml⁻¹). After extraction using a modified version of the method of Miller [29,
176 30], fatty acids were separated by gas chromatography and detected using the Microbial
177 Identification system (MIDI, Sherlock Version 6.1) [31].

178 For experimental genomic G+C content determination of LacT^T and LacV^T (done at the DSMZ
179 identification service), DNA was isolated and purified by chromatography on hydroxyapatite
180 [32] and the G+C content was determined by using HPLC [33].

181

182 Phylogenetic and genomic analyses

183 For 16S rRNA gene analyses, genomic DNA isolation from LacT^T and LacV^T cultures (5 mL)
184 and amplification by PCR (using 27F/1492r primer set; [34]) were performed as described
185 previously [35]. Sanger sequencing of the PCR product was performed at GATC Biotech AG
186 (Germany).

187 The 16S rRNA gene sequence was then compared with available sequences in the Genbank
188 database using the BLASTN search [36] and RDP tools [37] as RDP classifier [38]. All
189 evolutionary analyses were performed using MEGAX [39]. A multiple alignment was built
190 using the MUSCLE program [40]. Sequence positions with alignment uncertainty and gaps
191 were omitted from the analysis. Phylogenetic trees were reconstructed using three methods:

192 Neighbor-Joining, Maximum-Likelihood (Fig. 1) and Minimum Evolution methods [41-43].
193 The evolutionary distances were computed using the Kimura 2-parameter method [44]. The
194 analysis involved 15 nucleotide sequences having a total of 1291 positions in the final dataset.
195 Branch robustness was estimated by the non-parametric bootstrap procedure [45] (2000
196 replicates). The 16S rRNA sequences of strains LacT^T and LacV^T were deposited in Genbank
197 under accession number KJ159208 and KJ159207, respectively.
198 For whole-genome analyses, genomic DNA extraction of strains LacT^T and LacV^T was
199 performed as described previously [46]. A shotgun library was paired end sequenced on an
200 Illumina HiSeq 2000 instrument (2x100 bp) at the Marine Biological Laboratory, Woods Hole,
201 MA, USA. Reads of each data set were filtered, and high quality paired-end reads were *de novo*
202 assembled using SPAdes v. 3.5.0 (<http://cab.spbu.ru/software/spades/>) [47]. The obtained
203 whole genome draft sequences were annotated by using the NCBI Prokaryotic Genome
204 Annotation Pipeline (PGAP) and deposited at DDBJ/ENA/GenBank. For further comparative
205 analyses, the draft genomes were also annotated by the IMG/M expert review system and
206 incorporated in the IMG database (<https://img.jgi.doe.gov/>). The IMG Genome ID and
207 GenBank accession numbers of both investigated strains are listed in Table 1. A whole genome-
208 based taxonomic analysis of strains LacT^T and LacV^T genomes was performed using the
209 bioinformatics platform Type Strain Genome Server (TYGS) (Fig. S3).

210

211 Biogeography of the *Alkaliphilus* species from PBHF

212 The presence and abundance of the *Alkaliphilus* species in various serpentinite-hosted systems
213 worldwide were indirectly asserted by evaluating their genomes representation in the
214 metagenomes of these environments. A total of 27 “Whole Genome Sequencing” (WGS)
215 metagenomes from alkaline serpentinite-hosted ecosystems (Table S1) and 21 WGS

216 metagenomes from marine or terrestrial ecosystems (Table S2) were collected from NCBI
217 Sequence Read Archive (SRA) database with the *fastq-dump* program from NCBI SRA toolkit
218 (available at <https://trace.ncbi.nlm.nih.gov/Traces/sra/sra.cgi?view=software>) or with the
219 CURL program for datasets in the MG-RAST system. Metagenomic paired-end reads were
220 mapped to contigs of each *Alkaliphilus* genomes using the Anvi'o version 5.5 [48] snakemake
221 metagenomic workflow with the reference mode ([http://merenlab.org/2018/07/09/anvio-](http://merenlab.org/2018/07/09/anvio-snakemake-workflows/)
222 [snakemake-workflows/](http://merenlab.org/2018/07/09/anvio-snakemake-workflows/)). Raw paired-end reads were quality filtered using *illumina-utils* [49]
223 with the Minoche's algorithm [50]. Then, quality filtered reads were mapped to each
224 *Alkaliphilus* genome with Bowtie2 [51]. Results were normalized to the total quality filtered
225 reads of each sample to highlight the percent read mapped to each reference genome across the
226 serpentinite-hosted ecosystems. Additionally, results were visualized with Anvi'o version 5.5
227 to analyze the distribution of reads mapped along the genomes sequences [48].

228

229 **Results and discussion**

230 Strain isolation

231 Microbial growth was detected in enrichment cultures of chimney subsamples (ST08 and ST09)
232 after 2-week incubation in the dark at 30°C. Several colonies obtained after 7-day incubation at
233 30°C were creamy yellow, round with smooth edges and a diameter of 0.5–1 mm. Several
234 axenic cultures were obtained using the roll-tube method. Among them, strains LacT^T (from
235 ST08) and LacV^T (from ST09) were selected for further characterization.

236

237 Morphology

238 Strain LacT^T is a straight to slightly curved bacilli measuring $4.9 \pm 1.4 \mu\text{m}$ length and 0.3 ± 0.1
239 μm width during the exponential growth (Fig. 2A) and forms filamentous cells (up to 30 μm)

240 during stationary growth phase (Fig. 2B). Strain LacV^T is a straight, slightly shorter cell than
241 LacT^T ($3.7 \pm 0.8 \mu\text{m}$ length and $0.5 \pm 0.1 \mu\text{m}$ width; Fig. 3A). Both strains occur singly or in
242 pairs, were motile, and several lateral flagella were observed (Fig. 2C and Fig. 3B). The
243 presence of terminal endospores was observed by phase-contrast microscopy under stress
244 conditions or in prolonged cultures (not shown). The cell wall structure observed by
245 transmission microscopy revealed for both strains a Gram-positive structure with the presence
246 of a jagged peptidoglycan and a multilayered cell wall in the absence of an outer membrane
247 (Fig. 2D and Fig. 3C). Cell membrane staining also confirmed the Gram-positive structure of
248 both strains.

249

250 Phylogenetic affiliation

251 The 16S rRNA sequences of strains LacT^T and LacV^T share 96.4% identity between them and
252 93.3–96.3 % identity with those of the closest species belonging to the *Alkaliphilus* genus (order
253 *Clostridiales*), such as *A. peptidifermentans* Z-7036, *A. namsaraevii* X-07-2 and *A.*
254 *hydrothermalis* FatMR1 (95.7–96.3%), *A. transvaalensis* SAGM1 and *A. crotonatoxidans* B11-
255 2 (95.2–95.7%), and '*A. metalliredigens*' QYMF (not validated species), *A. oremlandii* OhILAs
256 and *A. halophilus* E2R (93.3–94.5%).

257

258 Genome features

259 The genome size and G+C content are 3.29 Mb and 36.0% for strain LacT^T and 3.06 Mb and
260 33.9 mol% for strain LacV^T which is consistent with the G+C content of the genus *Alkaliphilus*.
261 Strains LacT^T and LacV^T have a lower genome size than their closest phylogenetic neighbors,
262 *A. peptidifermentans* (4.45 Mb, 34.09% G+C) and *A. transvaalensis* (4.02 Mb, 33.95% G+C),
263 but close to *A. oremlandii* OhILAs (3.12 Mb, 36.3% G+C) [52]. The LacT^T strain genome

264 consisted of 83 contigs, with a N50 length of 157444 pb, and a total of 3257 genes were
265 predicted. Among them, 3183 were potential protein-coding genes (CDS). The LacV^T strain
266 genome consisted of 94 contigs, with a N50 length of 85817 pb, and a total of 3088 genes were
267 predicted of which 3035 were CDS. Both strains possess genes encoding flagella-related
268 proteins. The general genomic features of strains LacT^T and LacV^T are given in Table 1.

269 Value of average nucleotide identity (ANI) was calculated by using OrthoANI with usearch
270 (<http://www.ezbiocloud.net/tools/ani>). Digital DNA-DNA hybridization (dDDH) was
271 calculated using the Genome-to-Genome Distance Calculator (GGDC) 2.1
272 (<http://ggdc.dsmz.de/>) [53]. The ANI and dDDH values between LacT^T and LacV^T were found
273 to be 73.2% and 14.1%, i.e. far below the species boundaries of 95~96% and 70%, respectively,
274 recommended by Chun et al. [54] (Table 2). Additionally, ANI and dDDH values between the
275 genomes of both strains and other available *Alkaliphilus* genomes are also below the species
276 boundaries as presented in Table 2.

277

278 Chemotaxonomy

279 The major cellular fatty acids of strain LacT^T were C_{16:0} (50.5 % of the total), C_{18:0} (37.5 %),
280 C_{14:0} (6.9%) and C_{16:1 ω7c} (5.1 %); those of strain LacV^T were C_{16:0} (24.2%), C_{14:0} (20.3%), C_{18:0}
281 (14.4%), C_{16:1 ω7c} (11.1%) and C_{17:0 isoI / anteisoB} (6.6%) (Table S3). Genomic DNA of strains
282 LacT^T and LacV^T contained respectively 37.0 mol % and 36.3 mol % of G+C as determined by
283 HPLC at DSMZ. These values are 1.0 to 2.4% higher than those directly computed from the
284 genome sequences (see above).

285

286 Physiological characteristics and substrate utilization

287 Growth of strain LacT^T occurred in 0–7 % NaCl, at 25–37 °C and pH 7.5–10.6, with optimal
288 growth observed at 2 % NaCl, at 30 °C and at pH 8.2. Growth was observed for a maximum
289 level of 0.5% O₂ in the initial gas phase. Growth of strain LacV^T occurred in 0–8 % NaCl, at
290 20–40 °C and pH 8.2–10.0, with optimal growth at 2.5 % NaCl, at 30 °C and at pH 8.7. Growth
291 was observed for a maximum level of 0.1% O₂ concentration in the gas phase (Tables 3 and 4).
292 These data indicate that both strains are mesophilic, halotolerant, alkaliphilic and slightly
293 aerotolerant bacteria. The maximal growth rate obtained under optimal conditions was 0.035
294 h⁻¹ for strain LacT^T and 0.07 h⁻¹ for strain LacV^T.

295 Strain LacT^T was able to use yeast extract, peptone, casamino acids, glucose, maltose, sucrose
296 and crotonate. Casein, tryptone, cellobiose, fructose, galactose, lactose, mannose, ribose,
297 threhalose, xylose, starch, acetate, butyrate, citrate, lactate, fumarate, malate, pyruvate,
298 succinate, tartrate, acetone, ethanol, methanol, glycerol and butanol did not improve growth.

299 Strain LacV^T was able to use yeast extract, tryptone, peptone, casamino acids and crotonate but
300 none of the tested sugars. Arabinose, cellobiose, fructose, galactose, glucose, lactose, maltose,
301 mannose, melibiose, threhalose, raffinose, ribose, sucrose, xylose, acetate, butyrate, citrate,
302 lactate, formate, fumarate, malate, pyruvate, tartrate, succinate, and glycerol did not improve
303 growth. HPLC analysis confirmed the use of substrates.

304 When grown on sugars, the main metabolic product of strain LacT^T was acetate, accompanied
305 by H₂ and traces of CO₂. When grown on yeast extract, the main metabolic product of both
306 strains was acetate, and minor fermentation products were propionate, butyrate and hydrogen,
307 while strain LacT^T produced additionally isovalerate and CO₂. Crotonate was disproportionated
308 to acetate and butyrate by both strains.

309 None of the strain could grow by autotrophy on H₂:CO₂ (80:20, v:v) alone or on H₂:CO₂ plus
310 acetate (20 mM) as sole carbon sources. Additionally, no growth was observed on H₂:CO₂ or

311 on acetate (20 mM) combined each separately with Fe(OH)₃ (40 mM) or goethite α-
312 Fe(III)O(OH) (40 mM) as insoluble iron (III) oxyhydroxide, Fe (III) (EDTA), sulfate or nitrate
313 (20 mM). Both strains are thus strict organoheterotrophs.

314 The strains did not use any of the following electron acceptors tested in the BM medium with
315 yeast extract at 2.5 g/L as electron donor: fumarate, thiosulfate, sulfate, sulfite, nitrite, nitrate,
316 Fe(III)-citrate, Fe(III)-EDTA (20 mM each), Fe(OH)₃ (40 mM) or goethite α-Fe(III)O(OH) (40
317 mM) and elemental sulfur (10 g/L) suggesting they both have a strictly fermentative type of
318 energy metabolism. Thiosulfate slightly improved the growth of strain LacT^T but without H₂S
319 production, suggesting that thiosulfate is not used as an electron acceptor for respiration but
320 rather as a nutrient for anabolism.

321

322 Both strains LacT^T and LacV^T were able to use various proteinaceous compounds and casamino
323 acids, in accordance with their genomic data and similarly to *A. peptidifermentans* but unlike
324 *A. namsaraevii*, *A. hydrothermalis* and *A. transvaalensis* [55-58] (Table 3). Strain LacT^T
325 displayed a quite large range of substrate utilization compared to other *Alkaliphilus* and it is the
326 only one able to utilize glucose and sucrose among its closest relatives. It was also able to use
327 maltose similarly to *A. crotonatoxidans* [59]. On the contrary, strain LacV^T was not able to use
328 any tested sugars similarly to *A. namsaraevii*, *A. peptidifermentans* and *A. transvaalensis*.
329 Crotonate was disproportionated to acetate and butyrate by both strains LacT^T and LacV^T. The
330 disproportion of crotonate, as well as fermentation, could constitute an ecological advantage in
331 the anaerobic, highly reduced, high pH zones of serpentinizing environments known to be
332 depleted in oxidants needed for respiratory metabolisms [1]. With *A. crotonatoxidans*, both
333 strains LacT^T and LacV^T displayed slightly lower optimum growth pH compared to the closest
334 *Alkaliphilus*. Compared to *A. hydrothermalis* which is the previously isolated *Alkaliphilus*

335 specie from the Prony Bay Hydrothermal Field, strains LacT^T and LacV^T display higher NaCl
336 tolerance and prefer lower pH and temperature [55], suggesting a better adaptation to the
337 mixing-zone of seawater with the warm, alkaline and poorly salted hydrothermal fluid.
338 Based on the phenotypic characteristics and phylogenetic analyses described above, and given
339 the recommendations to recognize a new specie [54], strains LacT^T and LacV^T are considered
340 to represent novel species of the genus *Alkaliphilus*, for which the name *Alkaliphilus*
341 *serpentinus* sp. nov. and *Alkaliphilus pronyensis* sp. nov. are respectively proposed (Table 4).

342

343 Detection and abundance of *Alkaliphilus* species in serpentinizing hydrothermal systems

344 *Alkaliphilus* 16S rRNA gene sequences were consistently identified in several active chimneys
345 from submarine sites of PBHF over a thirteen year period (2005-2018), first in clone libraries
346 [11] and later by pyrosequencing of amplicons [18] or metagenomics [60]. Six *Alkaliphilus*
347 OTUs, mostly originating from ST09 sites, form a cluster together with strain LacV^T, and two
348 of them, from ST09 and ST07 sites, displayed more than 99% sequence identity (Fig. S2).
349 Moreover, two OTUs affiliated to the *Alkaliphilus* genus (96% identity over the complete 16S
350 rRNA sequence with either *A. metalliredigens* strain QMFY or with strain LacV^T) were found
351 in LCHF metagenomes, in which they represent each 3.94% and 0.56%, and 8.55% and 8.82%
352 of the 16S rRNA gene sequences of Marker 5 and Marker 3 metagenomes (LC.75 and LC.81),
353 respectively [61, 62]. The biogeography of the three *Alkaliphilus* species isolated from PBHF
354 was further evaluated by mapping the metagenomic paired-end reads from alkaline serpentinite-
355 hosted ecosystems (Table S1) and marine and continental ecosystems worldwide (Table S2), to
356 contigs of three *Alkaliphilus* species genomes (LacT^T, LacV^T and *A. hydrothermalis* FatMR1^T).
357 As expected, these novel *Alkaliphilus* genomes are well detected and distributed in PBHF
358 metagenomes, especially LacV^T and FatMR1^T which recruited, uniformly on the whole genome

359 sequences, between 0.02% up to near 1% of the reads depending on the sampled chimney. The
360 genome frequency was also dependent on the metagenomic reads coverage, even though the
361 mapping was normalized for all metagenomic samples used in this study. For example, LacV^T
362 and FatMR1^T genomes frequency in PBHF metagenomes are one order higher in the more
363 recent metagenomes [63] which also have nearly 20 times more reads (77 million reads vs 4
364 million reads) than the first metagenomes from ST09 chimneys (PBHF27 and PBHF28). This
365 is probably because rare or low abundant genotypes are just ‘missed’ in low coverage
366 metagenomic sequencing. In contrast, *Alkaliphilus* genomes are far less represented in other
367 metagenomes, including those from serpentinite-hosted ecosystems, where the recruited-reads
368 mapped only on a few, and limited regions, of the *Alkaliphilus* genomes. This could be
369 explained by the presence, in these metagenomes, of other *Clostridia* sharing core genes with
370 the novel *Alkaliphilus* genomes. Although *Alkaliphilus* species described so far have been
371 isolated from various ecosystems (anaerobic digester, soda lake, deep gold mine water, river
372 sediment, leachate pond) [52, 56-59, 64], the two novel *Alkaliphilus* species with *A.*
373 *hydrothermalis* previously described from PBHF [55] might represent indigenous member of
374 the PBHF ecosystem. Their prevalence and repeated detection in samples or cultures from all
375 but one of the active submarine chimneys from PBHF (they were not detected in the deepest
376 site ST012 of the Prony bay, sampled only once in 2011) are indicative of their ecological
377 significance. The fermentative metabolism of PBHF *Alkaliphilus* lead to the production of H₂
378 and organic acids (i.e., acetate, butyrate, propionate) *in situ* at concentrations that might sustain
379 the growth of microbial partners in syntrophy. This hypothetical partners could be
380 hydrogenotrophic autotrophs such as the elusive *Methanosarcinales*, that co-occur within the
381 same chimney samples, or hypothetical homoacetogens such as the candidate phyla
382 *Bipolaricaulota* (formely named *Acetothermia*) which are dominant in PBHF and LCHF [65]

383 but also prevalent in terrestrial (ophiolites) serpentinized ecosystems [10, 66]. Thus, the
384 *Methanosarcinales* could benefit, not only of the H₂ (also abiotically produced) but of the CO₂,
385 since the fluids are mostly depleted of DIC. Such an hypothesis has been recently formulated
386 to tentatively explain the metabolism of the Lost City *Methanosarcinales* (LCMS), unable to
387 use abiotically produced formate as dissolved carbon source, and thus would be dependent of
388 anaerobic formate-oxidizing bacteria, present in the same micro-niche, feeding them with CO₂
389 [67, 68].

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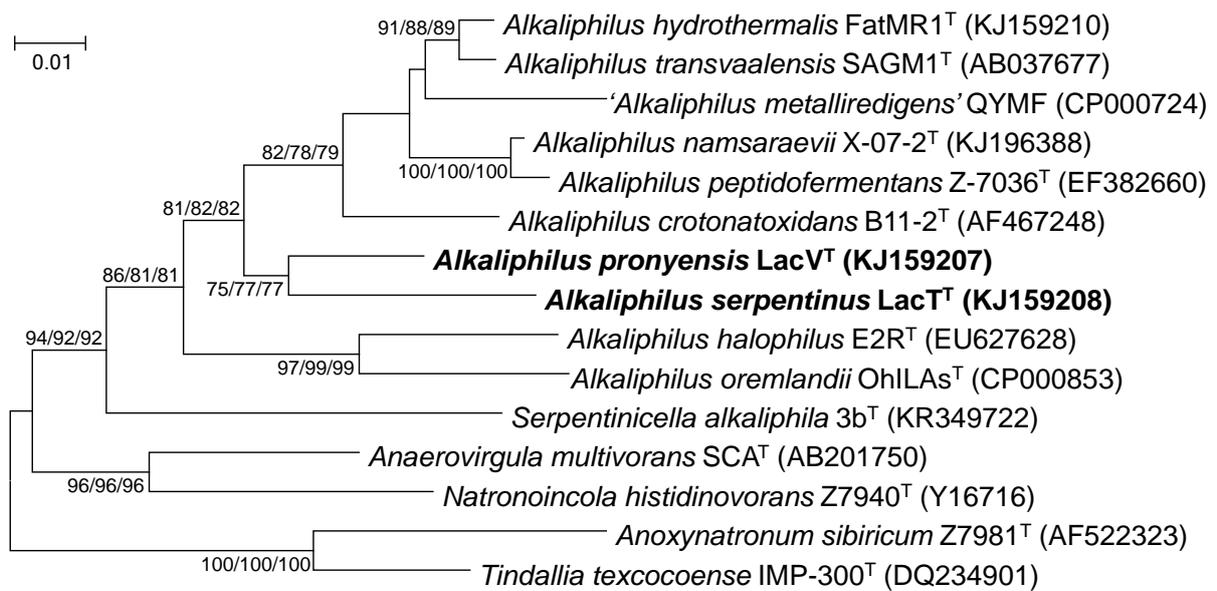
391

392 **Acknowledgments**

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394 Sustainable Development) and ANR MICROPRONY (N°19-CE02-0020-02). We greatly
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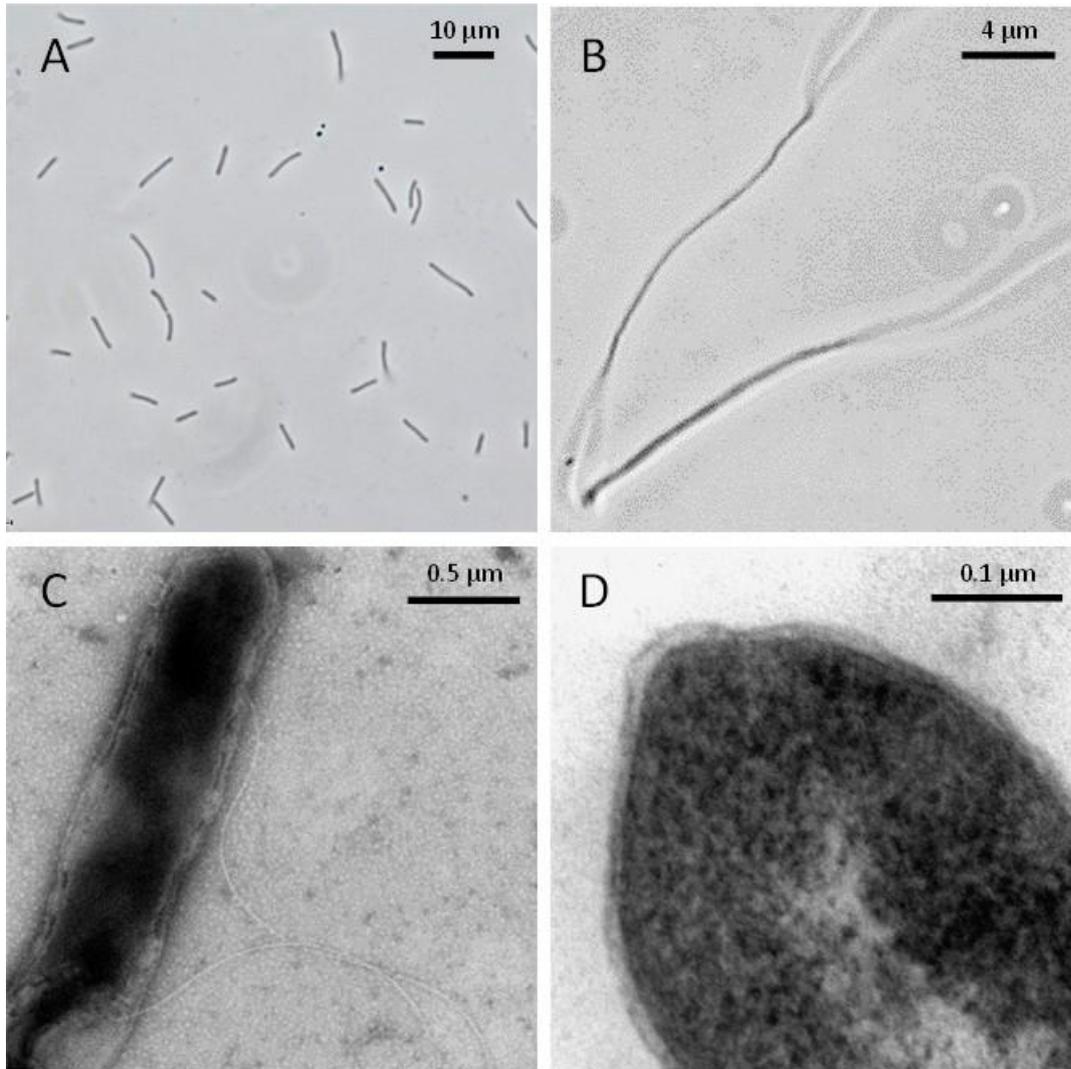


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404 **Fig. 1.** Maximum Likelihood phylogenetic tree based on 16S rRNA gene sequences showing
405 the position of strains LacT^T and LacV^T within *Alkaliphilus*. Sequences of type species in the
406 *Clostridiaceae* family are shown. The analysis involved a total of 1291 aligned nucleotide
407 positions in the final dataset. Bootstrap values (> 70 % shown) were obtained with the
408 maximum-likelihood / neighbor-joining / minimum evolution methods based on 2000
409 replicates. Bar: 0.01 substitutions per nucleotide.

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416 **Fig. 2.** Microphotographs of strain LacT^T showing (A) cells in exponential growth phase and

417 (B) elongated (filamentous) cells in stationary growth phase, both observed in phase contrast;

418 (C) peritrich lateral flagella and (D) thin section electron micrograph showing a jagged

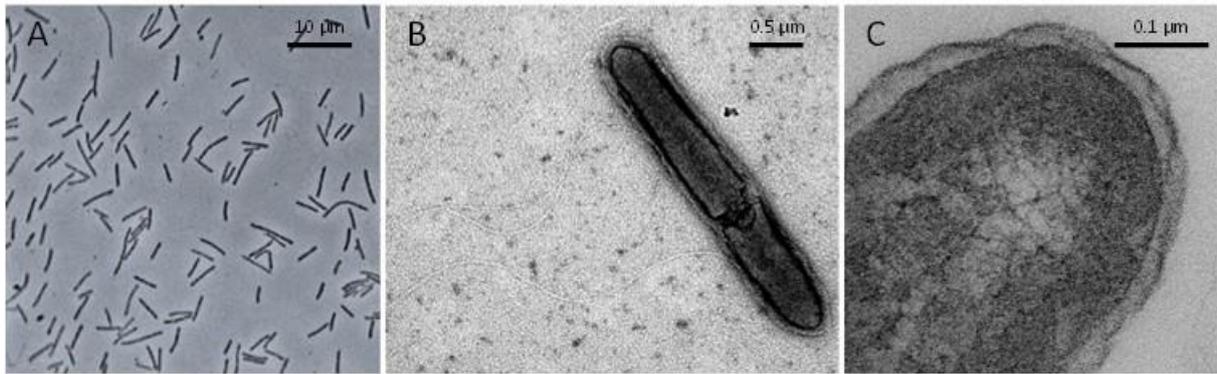
419 peptidoglycan and the absence of outer membrane, typical of a Gram-positive cell wall, both

420 observed in MET microscopy.

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426 **Fig. 3.** Microphotographs of strain LacV^T showing (A) cells in exponential growth phase
427 observed in phase contrast; (B) peritrich lateral flagella and (C) thin section electron
428 microscopy showing a jagged peptidoglycan and the absence of outer membrane, typical of a
429 Gram-positive cell wall, both observed in MET microscopy.

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433 **Table 1.** General features of the genomes of strains LacT^T and LacV^T.

Genome Name / Sample Name	<i>Alkaliphilus</i> sp. LacT^T	<i>Alkaliphilus</i> sp. LacV^T
GenBank accession number	WBZB000000000	WBZC000000000
IMG Genome ID	2639762554	2642422524
Genome Size (bp)	3291524	3061315
Gene Count	3257	3088
Scaffold count	83	94
GC (%)	36.00	33.87
Coding Base Count %	86.18	87.02
CDS Count	3183	3035
RNA Count	74	53
rRNA Count	1	2
tRNA Count	51	34

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438 **Table 2.** Digital DNA-DNA hybridization (dDDH: low left, green) and Average Nucleotide
 439 Identity (ANI: up right, red) values between strains LacT^T and LacV^T genomes and other
 440 *Alkaliphilus* species for which genome data were available: *A. peptidifermentans* Z-7036^T
 441 (=DSM 18978) (FMUS00000000.1), *A. transvaalensis* SAGM1^T (JHYF00000000.1), *A.*
 442 *metalliredigens* QYMF (CP000724) and *A. oremlandii* OhILAs^T (CP000853). dDDH were
 443 calculated using the Genome-to-Genome Distance Calculator (GGDC) 2.1
 444 (<http://ggdc.dsmz.de/>) [53]. ANI values were calculated using OrthoANI with usearch
 445 (<http://www.ezbiocloud.net/tools/ani>).

446

dDDH \ ANI (%)	<i>A. sp. LacT</i>	<i>A. sp. LacV</i>	<i>A. peptidifermentans</i>	<i>A. transvaalensis</i>	<i>A. metalliredigens</i>	<i>A. oremlandii</i>
<i>A. sp. LacT</i>		73.22	71.31	70.04	68.38	68.44
<i>A. sp. LacV</i>	14.10		68.74	70.54	68.97	68.74
<i>A. peptidifermentans</i>	13.40	15.50		70.83	70.20	69.16
<i>A. transvaalensis</i>	12.90	12.90	13.00		70.30	69.45
<i>A. metalliredigens</i>	12.60	12.70	12.90	13.00		69.80
<i>A. oremlandii</i>	12.60	12.70	12.70	12.80	13.00	

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451 **Table 3.** Differential phenotypic and genotypic characteristics between strains LacT^T, LacV^T and type strains of recognized *Alkaliphilus* species:
 452 *A. peptidifermentans* Z-7036^T [58], *A. namsaraevii* X-07-2^T [57], *A. hydrothermalis* FatMR1^T [24], *A. transvaalensis* SAGM1^T [56] and *A.*
 453 *crotonatoxidans* B11-2^T [59]. AFH: Amorphous ferric hydroxide, S₀: elemental sulfur, nd: not determined.

454

Characteristics	Strain LacT ^T	Strain LacV ^T	<i>A. peptidifermentans</i> Z-7036 ^T	<i>A. namsaraevii</i> X- 07-2 ^T	<i>A. hydrothermalis</i> FatMR1 ^T	<i>A. transvaalensis</i> SAGM1 ^T	<i>A. crotonatoxidans</i> B11-2 ^T
Origin	Prony Bay Hydrothermal Field	Prony Bay Hydrothermal Field	Soda lake Verkhnee Beloe	Transbaikal steppe lake	Prony Bay Hydrothermal Field	Gold Mine, South Africa	Wastewater treating anaerobic digester
Temperature growth range (opt.) (°C)	25–37 (30)	20–40 (30)	6–40 (35)	25–47 (30)	20–55 (37)	20–50 (40)	15–45 (37)
pH growth range (opt.)	7.5–10.6 (8.2)	8.2–10.0 (8.7)	7.5–9.7 (9.1)	7.0–10.7 (9.6–10.3)	7.5–10.5 (8.8–9.0)	8.5–12.5 (10.0)	5.5–9 (8.0)
NaCl growth range (opt.) (% w/v)	0–7 (2)	0–8 (2.5)	0–5 (2)	0.5–15 (4)	0–3 (0.2–0.5)	0.1–3.3 (0.5) ^a	0–7 (1.0) ^b
Electrons acceptors	–	–	crotonate, AFH, Fe(III)-EDTA, quinone, fumarate, thiosulfate	AFH, S ₀ , Fe(III) citrate	crotonate	crotonate ^c , S ₀ , fumarate, thiosulfate	crotonate
Crotonate dismutation	+	+	+	–	+	nd	+
Substrate use							
Casamino acids	+	+	+	–	–	–	nd
Glucose	+	–	–	–	–	–	–
Maltose	+	–	–	–	–	–	+
Sucrose	+	–	–	–	–	–	–
Main fermentation products	Acetate	Acetate	Acetate, Formate	Acetate	Acetate, H ₂ , CO ₂	nd	Acetate, Propionate
Major fatty acids	C16:0, C18:0	C16:0, C14:0, C18:0, C16:1ω7c	C16:0, C16:1ω7c, iso-C17, iso-C15, iso-C17:1	C16:1ω8, iso-C15:0, C14:0 3-OH, C16:0	C14:0, C16:0	iso-C15:0, iso-C17:0, iso-C15:1ω7c	C14:0, C16:0
G + C content (mol%)	36.0	33.9	33.8	32.6	37.1	36.4	30.6

455 ^a based on sea salts rather than NaCl addition; ^b from [64]; ^c from [59].

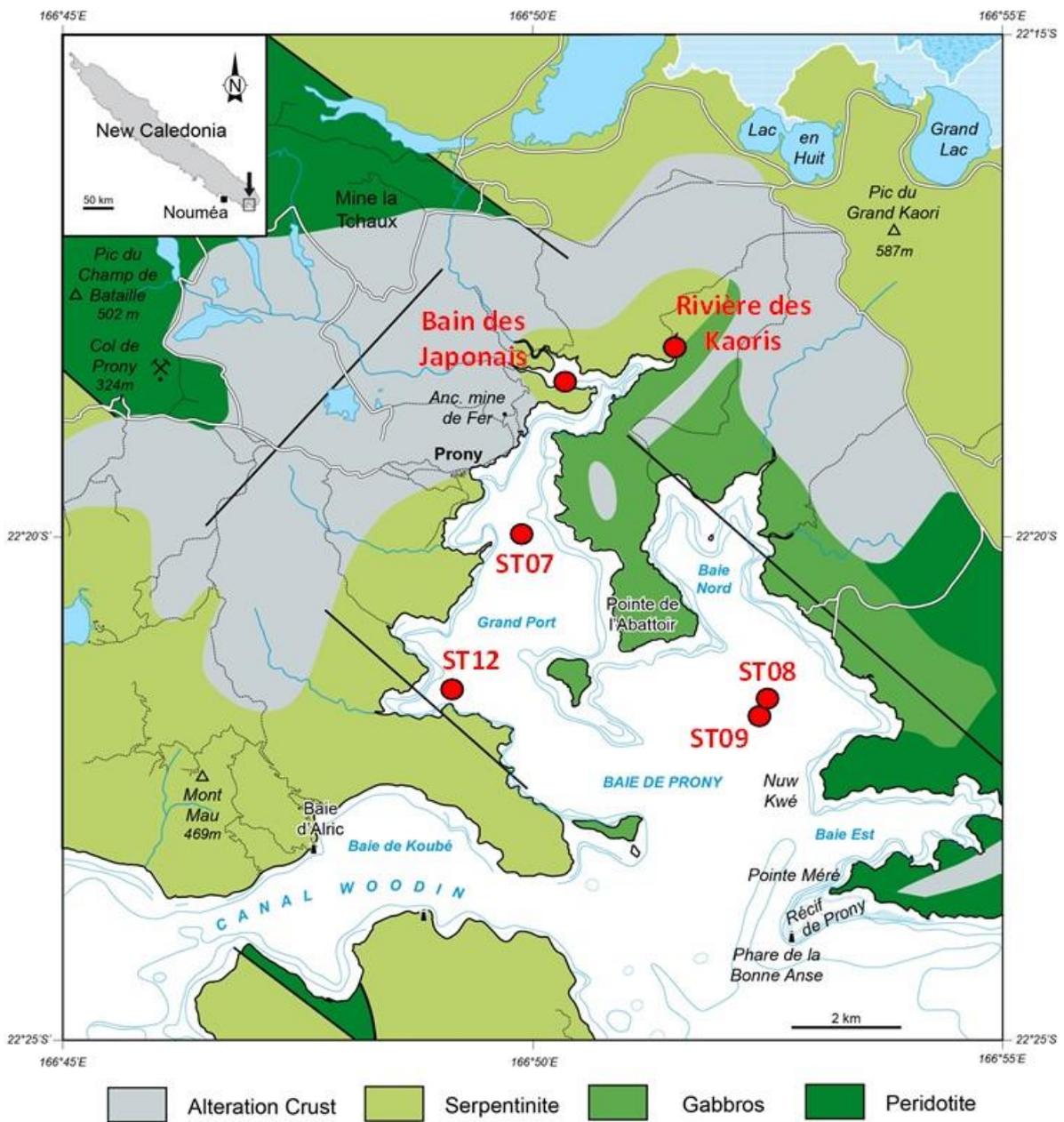
456 **Table 4.** Descriptions of *Alkaliphilus serpentinus* sp. nov. and *Alkaliphilus pronyensis* sp.
457 nov..

Genus name	<i>Alkaliphilus</i>	<i>Alkaliphilus</i>
Species name	<i>Alkaliphilus serpentinus</i>	<i>Alkaliphilus pronyensis</i>
Specific epithet	<i>serpentinus</i>	<i>pronyensis</i>
Species status	sp. nov.	sp. nov.
Species etymology	(ser.pen.ti'nus N.L. masc. adj. <i>serpentinus</i> from a serpentinizing environment	pro.ny.en'sis. N.L. masc. adj. <i>pronyensis</i> of or belonging to Prony Bay, where the type strain was isolated
Description of the new taxon and diagnostic traits	Mesophilic anaerobic bacterium. Rod cells ($4.9 \pm 1.4 \mu\text{m}$ length and $0.3 \pm 0.1 \mu\text{m}$ width during the exponential growth), forms filamentous cells (up to $30 \mu\text{m}$) during stationary growth phase. Gram-positive staining, forming terminal endospores, and motile by the means of lateral flagella. The temperature range for growth is 25–37°C, with an optimum at 30°C. The pH range for growth is 7.5–10.6, with an optimum at pH 8.2. Growth in the absence of NaCl (range 0–7 % NaCl, opt 2.0 %). Growth up to 0.5% O ₂ in the gas phase. Able to ferment yeast extract, peptone, casamino acids, glucose, maltose, and sucrose. Fermentation products from growth on yeast extract were acetate, propionate, butyrate, isovalerate, CO ₂ and H ₂ . Fermentation products from growth on glucose were acetate, H ₂ and CO ₂ (trace). Crotonate is disproportionated into acetate and butyrate. None of the following electron acceptors were used (fumarate, thiosulfate, sulfate, sulfite, nitrite, nitrate, Fe(III)-citrate, Fe(III)-EDTA, Fe(OH) ₃ , goethite α -Fe(III)O(OH) and elemental sulfur). The predominant fatty acids were C _{16:0} and C _{18:0} .	Mesophilic anaerobic bacterium. Rod cells (of $3.7 \pm 0.8 \mu\text{m}$ length and $0.5 \pm 0.1 \mu\text{m}$ width during the exponential growth), Gram-positive staining, forming terminal endospores, and motile by the means of lateral flagella. The temperature range for growth is 20–40°C, with an optimum at 30°C. The pH range for growth is 8.2–10.0, with an optimum at pH 8.7. Growth in the absence of NaCl (range 0–8 % NaCl, opt 2.5%). Growth up to 0.1% O ₂ in the gas phase. Able to ferment yeast extract, tryptone, peptone, casamino acids. None of the sugars tested were used. Fermentation products from growth on yeast extract were acetate, propionate, butyrate and H ₂ . Crotonate is disproportionated into acetate and butyrate. None of the following electron acceptors were used (fumarate, thiosulfate, sulfate, sulfite, nitrite, nitrate, Fe(III)-citrate, Fe(III)-EDTA, Fe(OH) ₃ , goethite α -Fe(III)O(OH) and elemental sulfur). The predominant fatty acids were C _{14:0} and C _{16:0} .
Country of origin	New Caledonia	New Caledonia
Region of origin	Prony Bay Hydrothermal Field	Prony Bay Hydrothermal Field
Source of isolation	Shallow submarine hydrothermal chimney of the site ST08 of the Prony Bay, New Caledonia	Shallow submarine hydrothermal chimney of the site ST09 of the Prony Bay, New Caledonia
Sampling date (dd/mm/yyyy)	11/2011	11/2011
Latitude (xx°xx'xx"N/S)	22°21'35"S	22°21'39"S
Longitude (xx°xx'xx"E/W)	166°52'50"E	166°52'46"E
16S rRNA gene accession nr.	KJ159208	KJ159207
Genome accession number [RefSeq; EMBL; ...]	WBZB00000000	WBZC00000000

Genome status	Incomplete	Incomplete
Genome size	3,290 kpb	3,060 kpb
GC mol% from genome analysis (from HPLC)	36.0% (37.0 %)	33.9% (36.3%)
Number of strains in study	1	1
Information related to the Nagoya Protocol	Permits obtained from Province Sud, Nouvelle-Calédonie, République Française	Permits obtained from Province Sud, Nouvelle-Calédonie, République Française
Designation of the Type Strain	LacT ^T	LacV ^T
Strain Collection Numbers	DSM100337 ^T , = JCM30643 ^T	DSM 100017 ^T , =JCM 30644 ^T

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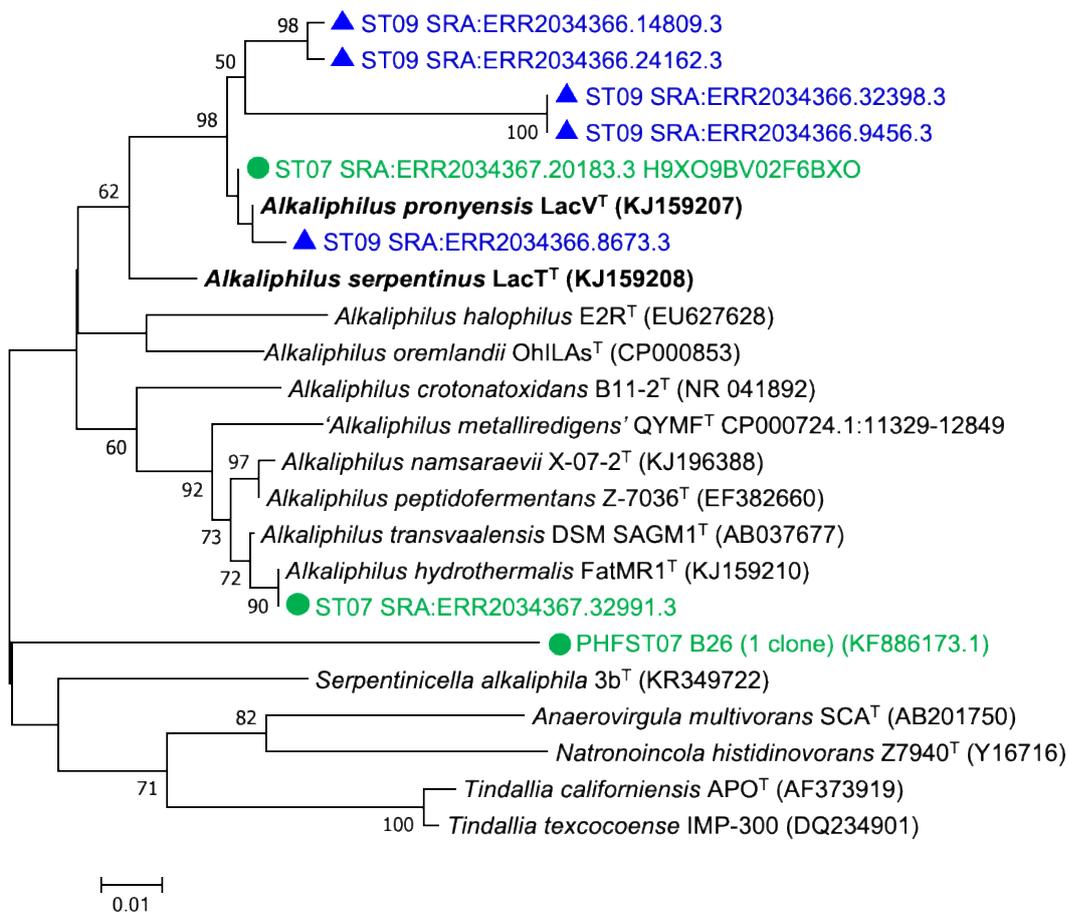
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462

463 **Fig. S1.** Simplified geological map of the Prony Bay, New Caledonia, South Pacific, modified from

464 [25, 69]. Strains LacT^T and LacV^T were isolated from samples of sites ST08 and ST09, respectively.

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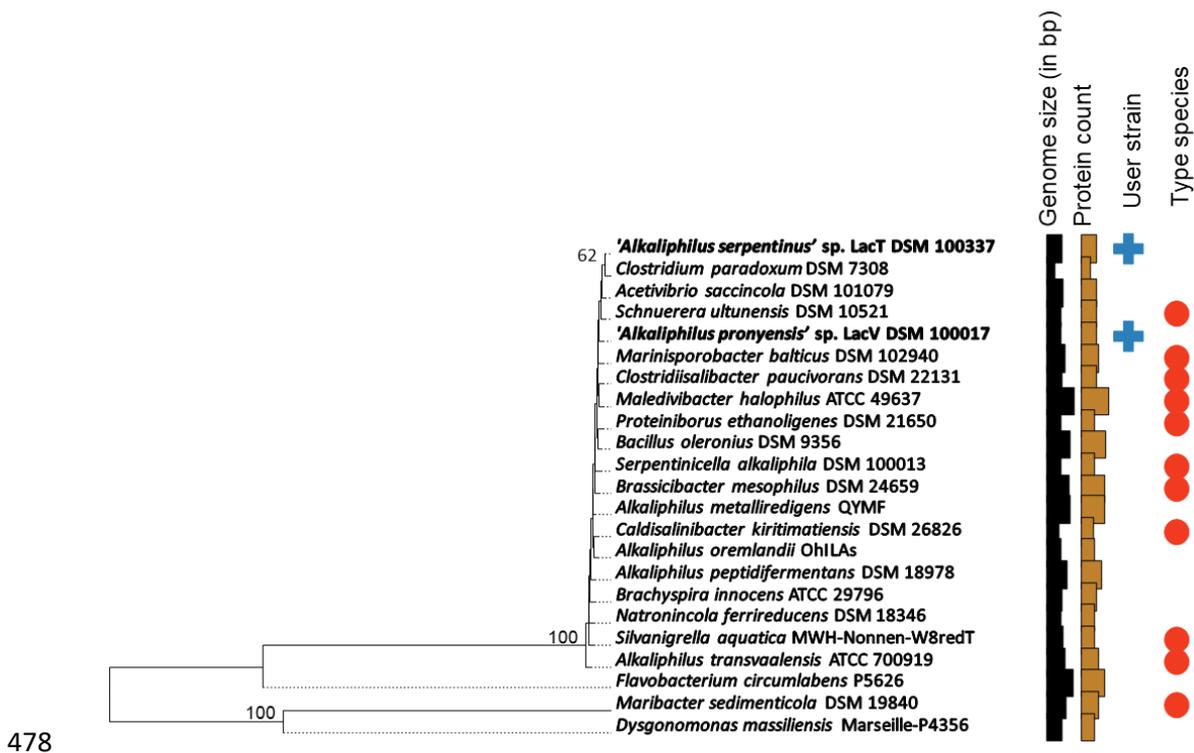
468

469 **Fig. S2.** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the
 470 position of strains LacT^T and LacV^T within *Alkaliphilus* and related environmental sequences.
 471 In blue (triangle), OTUs from PBHF ST09 site, in green (circle) OTUs from PBHF ST07 site.
 472 The analysis involved a total of 397 aligned nucleotide positions in the final dataset. Bootstrap
 473 values ($\geq 50\%$ shown) were based on 2000 replicates. Bar: 0.01 substitutions per nucleotide.

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479 **Fig. S3.** Whole genome-based taxonomic analysis of strains LacT^T and LacV^T genomes

480 performed using the bioinformatics platform Type (Strain) Genome Server (TYGS)

481 (<https://tygs.dsmz.de>) [70]. In brief, the TYGS analysis included a determination of the closest

482 type strain genomes by (i) comparison against all type strain genomes available in the TYGS

483 database *via* the MASH algorithm [71], and (ii) the closely related type strains determined *via*

484 the 16S rDNA gene sequences. For the phylogenomic inference, all pairwise comparisons

485 among the set of genomes were conducted using the Genome BLAST Distance Phylogeny

486 (GBDP) and accurate intergenomic distances inferred under the algorithm 'trimming' and

487 distance formula d5 [53]. The resulting intergenomic distances were used to infer a balanced

488 minimum evolution tree with branch support *via* FASTME 2.1.6.1 [72]. Branch support was

489 inferred from 100 pseudo-bootstrap replicates each. The trees were rooted at the midpoint [73].

490

491 **Table S1.** Mapping of metagenomic paired-end reads from alkaline serpentinite-hosted ecosystems to contigs of *Alkaliphilus* genomes of strains
 492 LacT^T, LacV^T and *A. hydrothermalis* FatMR1^T.

Location, country	Type	Sample	Accession (SRA or MG-Rast)	Ref.	Total pairs passed (QC)	Alk_LacT	Alk_LacT (% mapped)	Alk_LacV	Alk_LacV (% mapped)	Alk_FatMR1	Alk_FatMR1 (% mapped)
Prony Bay, New Caledonia	Hydrothermal chimney	PBHF27 (ST09)	SRR1636516	[60]	4747529	47	0.0010	1704	0.0359	1067	0.0225
		PBHF28 (ST09)	SRR1636517		6012777	8	0.0001	1151	0.0191	1574	0.0262
		BdJ-11	NP	[63]	68376909	117966	0.1725	120271	0.1759	107973	0.1579
		BdJ-14			77737610	256760	0.3303	204727	0.2634	691304	0.8893
		RdK-14			68115190	123697	0.1816	127390	0.1870	114595	0.1682
		ST07-11			68444258	130824	0.1911	135275	0.1976	119136	0.1741
		ST07-14			63035475	123890	0.1965	225332	0.3575	102358	0.1624
Lost City hydrothermal field, MAR	Hydrothermal chimney	LCHF75	SRR1636508	[67]	12911379	69	0.0005	2517	0.0195	3605	0.0279
		LCHF81	SRR1636509		12484886	46	0.0004	2206	0.0177	3045	0.0244
Old City hydrothermal field, SWIR	Hydrothermal chimney	BaC	SRR9822619	[65]	19648677	3	0.0000	1447	0.0074	1905	0.0097
		PiMo	SRR9822616		19440539	0	0.0000	1066	0.0055	1425	0.0073
		Chan	SRR9822617		20478069	17	0.0001	1929	0.0094	2601	0.0127
		OCT	SRR9822614		21034520	6	0.0000	861	0.0041	1246	0.0059
		ToMo	SRR9822615		109686580	4	0.0000	3752	0.0034	5277	0.0048
The Cedars ophiolite, USA	Spring water	BS5B_11	DRR093101	[74]	24714554	14	0.0001	2309	0.0093	4586	0.0186
		BS5B_12	DRR093102		22551823	19	0.0001	2491	0.0110	5094	0.0226
		GPS1_11	DRR093103		26117910	18	0.0001	1902	0.0073	3337	0.0128
		GPS1_12	DRR093104		22036872	7	0.0000	1451	0.0066	2607	0.0118
Voltri Massif, Italy	Spring water	BR2_12	SRR2058405	[75]	14713490	1	0.0000	2030	0.0138	2905	0.0197
		BR2_13	SRR1636510		7616762	3	0.0000	736	0.0097	1192	0.0156
		GOR_12	SRR2058407		18290914	42	0.0002	1663	0.0091	3027	0.0165
		GOR_13	SRR1636513		25243838	105	0.0004	4276	0.0169	7465	0.0296

Cabeço de Vide aquifer, Portugal	Groundwater from a borehole	CVA	SRR1636515	NP	5871482	12	0.0002	1800	0.0307	2436	0.0259
Coast range ophiolite microbial observatory, USA	Groundwater from well	CSW1.1	4569549,3	[76]	9108623	1	0.0000	756	0.0083	1975	0.0217
		CSW1.3	4569551,3		16893824	345	0.0020	7839	0.0464	10688	0.0633
		QV1.1	4569550,3		15290683	2	0.0000	9805	0.0641	16685	0.1091
		QV1.2	4569552,3		12997149	730	0.0056	3064	0.0236	6947	0.0535
Santa Elena ophiolite, Costa Rica	Spring water	CM_SE_9	SRR4101185	[77]	7416565	0	0.0000	998	0.0135	1522	0.0205
		SE_9	SRR1636511	NP	51813498	216	0.0004	4387	0.0085	6495	0.0125
Peninsula Iberica	Deep aquifer	AC3	SRR6347719	NP	15897509	25	0.0002	3237	0.0204	3776	0.0238
		EBD	SRR6347720		12663159	116	0.0009	2600	0.0205	3774	0.0298
		EBPU	SRR6347718		12555955	22	0.0002	1227	0.0098	1683	0.0134

493 NP; Not published.

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495

496 **Table S2.** Mapping of metagenomic paired-end reads from marine or terrestrial ecosystems to contigs of *Alkaliphilus* genomes of strains LacT^T,497 LacV^T and *A. hydrothermalis* FatMR1^T.

Location, country	Type	Sample	Accession (SRA or MG-Rast)	Ref.	Total pairs passed (QC)	Alk_LacT	Alk_LacT (% mapped)	Alk_LacV	Alk_LacV (% mapped)	Alk_FatMR1	Alk_FatMR1 (% mapped)
Axial Seamount, Juan de Fuca ridge	Basaltic hydrothermal fluids	Mkr33	ERR694199- ERR694206	[78]	25725851	6	0.0000	6210	0.0241	88417	0.3437
		Mkr113	ERR694207- ERR694213		22624331	12	0.0001	3336	0.0147	4464	0.0197
Piccard	Basaltic hydrothermal fluids	HC	ERR1662208- ERR1662232	[79]	78203317	24	0,0000	68060	0,0870	90624	0,1159
		MkrX19	ERR1662466- ERR1662503		141073647	2	0,0000	33365	0,0237	45580	0,0323
		SC	ERR1662233- ERR1662244		38058339	0	0,0000	208229	0,5471	295789	0,7772
		SG2	ERR868087- ERR868115		104273127	0	0,0000	21601	0,0207	30254	0,0290
Von Damm	Serpentinite-hosted hydrothermal fluids (non-alkaline)	GC	ERR868031- ERR868053	[79]	77403857	14	0.0000	18153	0.0235		
		MH	ERR1662199- ERR1662207		26539513	2	0.0000	6105	0.0230	8469	0.0319
		OMT	ERR1662181- ERR1662198		53972022	0	0.0000	17338	0.0321	25239	0.0468
		Rav2	ERR1662393- ERR1662413		64064672	0	0.0000	2926	0.0046	4566	0.0071
		SH	ERR1662414- ERR1662442		104733336	4	0.0000	14418	0.0138	20388	0.0195
Guaymas Basin	Hydrothermal sediments	4484 shallow	SRR6301444	[80]	233093488	306344	0.1314	312308	0.1340	284008	0.1218
		4484 deep	SRR6301445		179762089	462149	0.2571	470732	0.2619	426607	0.2373
		4572 shallow	SRR6301446		203371756	287320	0.1413	313182	0.1540	292335	0.1437
		4572 deep	SRR6301447		246429337	386166	0.1567	402130	0.1632	368436	0.1495
		S1	SRR7716469	[81]	40523149	13	0.0000	2556	0.0063	3494	0.0086
Gulf of Mexico	Methane seep sediments	S2	SRR7716470		69582704	0	0.0000	3356	0.0048	3999	0.0057

		S3	SRR7716471		16553149	0	0,0000	1548	0,0094	3833	0,0232
South Africa	Fracture fluids	MM51	SRR1633224	Not published	20525179	69	0,0003	5997	0,0292	7810	0,0381
Gold mine		NO14	SRR1633225		29027742	156	0,0005	14094	0,0486	22127	0,0762
		TT10	SRR1633226		16553149	0	0,0000	1548	0,0094	3833	0,0232

NP; Not published.

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502 **Table S3.** Fatty acids compositions of strains LacT^T and LacV^T and type strains of related
 503 *Alkaliphilus* species: *A. peptidifermentans* Z-7036^T [58], *A. namsaraevii* X-07-2^T [57], *A.*
 504 *hydrothermalis* FatMR1^T [24], *A. transvaalensis* SAGM1^T [56] and *A. crotonatoxidans* B11-2^T
 505 [59]. The two dominant fatty acids are indicated in bold for each strain. - ; not detected.

Fatty acids	LacT ^T	LacV ^T	Z-7036 ^T	X-07-2 ^T	FatMR1 ^T	SAGM1 ^T	B11-2 ^T
C _{14:0}	6.9	20.3	-	10.9	44.2	1.7	45.6
C_{14:0}3-OH	-	-	-	18.6	-	-	-
iso-C _{15:0}	-	4.9	10.5	36.2	4.9	51.6	7.1
C _{16:0}	50.5	24.2	20.1	18.6	14.3	3.9	17.4
C_{16:1}7c	5.1	11.1	14.5	-	9	1.9	2.4
C _{16:1} 8	-	-	-	37.0	-	-	-
iso-C _{17:0}	-	0.6	11.3	-	-	12.2	1.1
C _{17:0} isoI / anteisoB	-	6.8	-	-	-	-	-
C _{18:0}	37.5	14.4	1.9	4.2	-	2.0	0.8

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References

- 509 [1] Schrenk, M.O., Brazelton, W.J., Lang, S.Q. 2013, Serpentinization, carbon, and deep life, in: R.M.
510 Hazen, A.P. Jones, J.A. Baross (Eds.) *Rev Mineral Geochem*, pp. 575-606.
- 511 [2] Martin, W., Baross, J., Kelley, D., Russell, M.J. (2008) Hydrothermal vents and the origin of life. *Nat*
512 *Rev Micro.* 6, 805-814.
- 513 [3] Suzuki, S., Ishii, S.i., Wu, A., Cheung, A., Tenney, A., Wanger, G., Kuenen, J.G., Nealson, K.H. (2013)
514 Microbial diversity in The Cedars, an ultrabasic, ultrareducing, and low salinity serpentinizing
515 ecosystem. *Proc Natl Acad Sci U S A.* 110, 15336-15341.
- 516 [4] Woycheese, K., Meyer-Dombard, D.A.R., Cardace, D., Argayosa, A., Arcilla, C. (2015) Out of the dark:
517 Transitional subsurface-to-surface microbial diversity in a terrestrial serpentinizing seep (Manleluag,
518 Pangasinan, the Philippines). *Front Microbiol.* 6.
- 519 [5] Brazelton, W.J., Morrill, P.L., Szponar, N., Schrenk, M.O. (2013) Bacterial communities associated
520 with subsurface geochemical processes in continental serpentinite springs. *Appl Environ Microbiol.* 79,
521 3906-3916.
- 522 [6] Tiago, I., Veríssimo, A. (2013) Microbial and functional diversity of a subterrestrial high pH
523 groundwater associated to serpentinization. *Environ Microbiol.* 15, 1687-1706.
- 524 [7] Itävaara, M., Nyysönen, M., Kapanen, A., Nousiainen, A., Ahonen, L., Kukkonen, I. (2011)
525 Characterization of bacterial diversity to a depth of 1500 m in the Outokumpu deep borehole,
526 Fennoscandian Shield. *FEMS Microbiol Ecol.* 77, 295-309.
- 527 [8] Brazelton, W.J., Schrenk, M.O., Kelley, D.S., Baross, J.A. (2006) Methane- and sulfur-metabolizing
528 microbial communities dominate the Lost City Hydrothermal Field Ecosystem. *Appl Environ Microbiol.*
529 72, 6257-6270.
- 530 [9] Kelley, D.S., Karson, J.A., Früh-Green, G.L., Yoerger, D.R., Shank, T.M., Butterfield, D.A., Hayes, J.M.,
531 Schrenk, M.O., Olson, E.J., Proskurowski, G., Jakuba, M., Bradley, A., Larson, B., Ludwig, K., Glickson,
532 D., Buckman, K., Bradley, A.S., Brazelton, W.J., Roe, K., Elend, M., Delacour, A., Bernasconi, S.M., Lilley,
533 M.D., Baross, J.A., Summons, R.E., Sylva, S.P. (2005) A serpentinite-hosted ecosystem: the Lost City
534 Hydrothermal Field. *Science.* 307, 1428-1434.
- 535 [10] Postec, A., Quemeneur, M., Bes, M., Mei, N., Benaissa, F., Payri, C., Pelletier, B., Monnin, C.,
536 Guentas-Dombrowsky, L., Ollivier, B., Gerard, E., Pisapia, C., Gerard, M., Menez, B., Erauso, G. (2015)
537 Microbial diversity in a submarine carbonate edifice from the serpentinizing hydrothermal system of
538 the Prony Bay (New Caledonia) over a 6-year period. *Front Microbiol.* 6, 857.
- 539 [11] Quémeneur, M., Bes, M., Postec, A., Mei, N., Hamelin, J., Monnin, C., Chavagnac, V., Payri, C.,
540 Pelletier, B., Guentas-Dombrowsky, L., Gérard, M., Pisapia, C., Gérard, E., Ménez, B., Ollivier, B., Erauso,
541 G. (2014) Spatial distribution of microbial communities in the shallow submarine alkaline hydrothermal
542 field of the Prony Bay, New Caledonia. *Environ Microbiol Rep.* 6, 665-674.
- 543 [12] Brazelton, W.J., Nelson, B., Schrenk, M.O. (2012) Metagenomic evidence for H₂ oxidation and H₂
544 production by serpentinite-hosted subsurface microbial communities. *Front Microbiol.* 2.
- 545 [13] Vignais, P.M., Billoud, B., Meyer, J. (2001) Classification and phylogeny of hydrogenases. *FEMS*
546 *Microbiol Rev.* 25, 455-501.
- 547 [14] Bath, A.H., Christofi, N., Neal, C., Philip, J.C., Cave, M.R., McKinley, I.G., Berner, U. (1987) Trace
548 element and microbiological studies of alkaline groundwaters in Oman, Arabian Gulf: a natural
549 analogue for cement pore-waters. Report of the Fluid Processes Research Group. Report FLPU 87-2.
550 British Geological Survey, Keyworth, United Kingdom.
- 551 [15] Meyer-Dombard, D.A.R., Woycheese, K.M., Yargicoglu, E.N., Cardace, D., Shock, E.L., Güleçal-
552 Pektas, Y., Temel, M. (2015) High pH microbial ecosystems in a newly discovered, ephemeral,
553 serpentinizing fluid seep at Yanartaş (Chimaera), Turkey. *Front Microbiol.* 5.

554 [16] Tiago, I., Chung, A.P., Verissimo, A. (2004) Bacterial diversity in a nonsaline alkaline environment:
555 heterotrophic aerobic populations. *Appl Environ Microbiol.* 70, 7378-7387.

556 [17] Tiago, I., Pires, C., Mendes, V., Morais, P.V., da Costa, M.S., Verissimo, A. (2006) *Bacillus foraminis*
557 sp. nov., isolated from a non-saline alkaline groundwater. *Int J Syst Evol Microbiol.* 56, 2571-2574.

558 [18] Frouin, E., Bes, M., Ollivier, B., Quéméneur, M., Postec, A., Debroas, D., Armougom, F., Erauso, G.
559 (2018) Diversity of rare and abundant prokaryotic phylotypes in the Prony Hydrothermal Field and
560 comparison with other serpentinite-hosted ecosystems. *Front Microbiol.* 9, 102.

561 [19] Pisapia, C., Gerard, E., Gerard, M., Lecourt, L., Lang, S.Q., Pelletier, B., Payri, C.E., Monnin, C.,
562 Guentas, L., Postec, A., Quemeneur, M., Erauso, G., Menez, B. (2017) Mineralizing Filamentous Bacteria
563 from the Prony Bay Hydrothermal Field Give New Insights into the Functioning of Serpentinization-
564 Based Subseafloor Ecosystems. *Front Microbiol.* 8, 57.

565 [20] Ben Aissa, F., Postec, A., Erauso, G., Payri, C., Pelletier, B., Hamdi, M., Ollivier, B., Fardeau, M.-L.
566 (2014) Characterization of *Vallitalea pronyensis* sp. nov., a novel marine bacterium, isolated from an
567 alkaline hydrothermal chimney in Prony Bay, New Caledonia. *Int J Syst Evol Microbiol.* 64, 1160-1165.

568 [21] Bes, M., Merrouch, M., Joseph, M., Quéméneur, M., Payri, C., Pelletier, B., Ollivier, B., Fardeau,
569 M.-L., Erauso, G., Postec, A. (2015) *Acetoanaerobium pronyense* sp. nov., an anaerobic alkaliphilic
570 bacterium isolated from the Prony alkaline Hydrothermal Field in New Caledonia. *Int J Syst Evol*
571 *Microbiol.* 65, 2574–2580.

572 [22] Mei, N., Zergane, N., Postec, A., Erauso, G., Oilier, A., Payri, C., Pelletier, B., Fardeau, M.-L., Ollivier,
573 B., Quemeneur, M. (2014) Fermentative hydrogen production by a new alkaliphilic *Clostridium* sp
574 (strain PROH2) isolated from a shallow submarine hydrothermal chimney in Prony Bay, New Caledonia.
575 *Int J Hydrog Energy.* 39, 19465-19473.

576 [23] Mei, N., Postec, A., Erauso, G., Joseph, M., Pelletier, B., Payri, C., Ollivier, B., Quéméneur, M. (2016)
577 *Serpentinicella alkaliphila* gen. nov., sp. nov., a novel alkaliphilic anaerobic bacterium isolated from the
578 serpentinite-hosted Prony hydrothermal field, New Caledonia. *Int J Syst Evol Microbiol.* 66, 4464-4470.

579 [24] Ben Aissa, F., Postec, A., Erauso, G., Payri, C., Pelletier, B., Hamdi, M., Fardeau, M.-L., Ollivier, B.
580 (2015) Characterization of *Alkaliphilus hydrothermalis* sp. nov., a novel alkaliphilic anaerobic
581 bacterium, isolated from a carbonaceous chimney of the Prony hydrothermal field, New Caledonia.
582 *Extremophiles.* 19, 183-188.

583 [25] Monnin, C., Chavagnac, V., Boulart, C., Ménez, B., Gérard, M., Gérard, E., Pisapia, C., Quéméneur,
584 M., Erauso, G., Postec, A., Guentas-Dombrowski, L., Payri, C., Pelletier, B. (2014) Fluid chemistry of the
585 low temperature hyperalkaline hydrothermal system of Prony Bay (New Caledonia). *Biogeosciences.*
586 11, 5687-5706.

587 [26] Hungate, R.E. 1969, A roll tube method for the cultivation of strict anaerobes, in: J.R. Norris, D.W.
588 Ribbons (Eds.) *Meth Microbiol*, Academic Press, London, pp. 117–132.

589 [27] Cord-Ruwisch, R. (1985) A quick method for determination of dissolved and precipitated sulfides
590 in cultures of sulfate-reducing bacteria. *J Microbiol Met* 4, 33-36.

591 [28] Lovley, D.R., Phillips, E.J. (1986) Availability of ferric iron for microbial reduction in bottom
592 sediments of the freshwater tidal potomac river. *Appl environ microbiol.* 52, 751-757.

593 [29] Kuykendall, L.D., Roy, M.A., O'Neill, J.J., Devine, T.E. (1988) Fatty acids, antibiotic resistance, and
594 deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Evol Bact.* 38, 358-361.

595 [30] Miller, L.T. (1982) Single derivatization method for routine analysis of bacterial whole-cell fatty
596 acid methyl esters, including hydroxy acids. *J Clin Microbiol.* 16, 584-586.

597 [31] Sasser, M. (1990) Identification of bacteria by gas chromatography of cellular fatty acids. MIDI
598 Technical Note 101. Microbial ID, Inc., Newark, DE, USA.

599 [32] Cashion, P., Holder-Franklin, M.A., McCully, J., Franklin, M. (1977) A rapid method for the base
600 ratio determination of bacterial DNA. *Anal Biochem.* 81, 461-466.

601 [33] Mesbah, M., Premachandran, U., Whitman, W.B. (1989) Precise measurement of the G+C content
602 of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol.* 39, 159-167.

603 [34] Lane, D.J. 1991, 16S/23S rRNA sequencing., in: E. Stackebrandt, M. Goodfellow (Eds.) Nucleic acid
604 techniques in bacterial systematics, John Wiley and Sons, New York, pp. 115-175.

605 [35] Bes, M., Merrouch, M., Joseph, M., Quemeneur, M., Payri, C., Pelletier, B., Ollivier, B., Fardeau,
606 M.L., Erauso, G., Postec, A. (2015) *Acetoanaerobium pronyense* sp. nov., an anaerobic alkaliphilic
607 bacterium isolated from a carbonate chimney of the Prony Hydrothermal Field (New Caledonia). Int J
608 Syst Evol Microbiol. 65, 2574-2580.

609 [36] Zhang, Z., Schwartz, S., Wagner, L., Miller, W. (2000) A Greedy Algorithm for Aligning DNA
610 Sequences. J Comput Biol. 7, 203-214.

611 [37] Cole, J.R., Wang, Q., Fish, J.A., Chai, B., McGarrell, D.M., Sun, Y., Brown, C.T., Porras-Alfaro, A.,
612 Kuske, C.R., Tiedje, J.M. (2014) Ribosomal Database Project: data and tools for high throughput rRNA
613 analysis. Nucl Acids Res. 42, D633-D642.

614 [38] Wang, Q., Garrity, G.M., Tiedje, J.M., Cole, J.R. (2007) Naïve bayesian classifier for rapid
615 assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 73, 5261-
616 5267.

617 [39] Kumar, S., Stecher, G., Li, M., Niyaz, C., Tamura, K. (2018) MEGA X: Molecular Evolutionary
618 Genetics Analysis across Computing Platforms. Mol Biol Evol. 35, 1547-1549.

619 [40] Edgar, R.C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput.
620 Nucl Acids Res. 32, 1792-1797.

621 [41] Felsenstein, J. (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J
622 Mol Evol. 17, 368-376.

623 [42] Rzhetsky, A., Nei, M. (1992) Statistical properties of the ordinary least-squares, generalized least-
624 squares, and minimum-evolution methods of phylogenetic inference. J Mol Evol. 35, 367-375.

625 [43] Saitou, N., Nei, M. (1987) The neighbor-joining method: a new method for reconstructing
626 phylogenetic trees. Mol Biol Evol. 4, 406-425.

627 [44] Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions
628 through comparative studies of nucleotide sequences. J Mol Evol. 16, 111-120.

629 [45] Felsenstein, J. (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution.
630 39, 783-791.

631 [46] Quémeneur, M., Erauso, G., Frouin, E., Zeghal, E., Vandecasteele, C., Ollivier, B., Tamburini, C.,
632 Garel, M., Ménez, B., Postec, A. (2019) Hydrostatic pressure helps to cultivate an original anaerobic
633 bacterium from the Atlantis Massif Subseafloor (IODP Expedition 357): *Petrocella atlantisensis* gen.
634 nov. sp. nov. Front Microbiol. 10.

635 [47] Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Lesin, V.M.,
636 Nikolenko, S.I., Pham, S., Prjibelski, A.D., Pyshkin, A.V., Sirotkin, A.V., Vyahhi, N., Tesler, G., Alekseyev,
637 M.A., Pevzner, P.A. (2012) SPAdes: a new genome assembly algorithm and its applications to single-
638 cell sequencing. J Comput Biol. 19, 455-477.

639 [48] Eren, A., Esen, Ö., Quince, C., Vineis, J., Morrison, H., Sogin, M., Delmont, T. (2015) Anvi'o: an
640 advanced analysis and visualization platform for 'omics data. PeerJ. 3:e1319.

641 [49] Eren, A.M., Vineis, J.H., Morrison, H.G., Sogin, M.L. (2013) A Filtering Method to Generate High
642 Quality Short Reads Using Illumina Paired-End Technology. PLOS ONE. 8, e66643.

643 [50] Minoche, A.E., Dohm, J.C., Himmelbauer, H. (2011) Evaluation of genomic high-throughput
644 sequencing data generated on Illumina HiSeq and Genome Analyzer systems. Genome Biol. 12, R112.

645 [51] Langmead, B., Salzberg, S.L. (2012) Fast gapped-read alignment with Bowtie 2. Nat Methods. 9,
646 357-359.

647 [52] Hwang, C., Copeland, A., Lucas, S., Lapidus, A., Barry, K., Detter, J.C., Glavina Del Rio, T., Hammon,
648 N., Israni, S., Dalin, E., Tice, H., Pitluck, S., Chertkov, O., Brettin, T., Bruce, D., Han, C., Schmutz, J.,
649 Larimer, F., Land, M.L., Hauser, L., Kyrpides, N., Mikhailova, N., Ye, Q., Zhou, J., Richardson, P., Fields,
650 M.W. (2016) Complete genome sequence of *Alkaliphilus metalliredigens* strain QYMF, an alkaliphilic

651 and metal-reducing bacterium isolated from borax-contaminated leachate ponds. Genome announc.
652 4, e01226-01216.

653 [53] Meier-Kolthoff, J.P., Auch, A.F., Klenk, H.-P., Göker, M. (2013) Genome sequence-based species
654 delimitation with confidence intervals and improved distance functions. BMC Bioinformatics. 14, 60.

655 [54] Chun, J., Oren, A., Ventosa, A., Christensen, H., Arahal, D.R., da Costa, M.S., Rooney, A.P., Yi, H.,
656 Xu, X.-W., De Meyer, S., Trujillo, M.E. (2018) Proposed minimal standards for the use of genome data
657 for the taxonomy of prokaryotes. Int J Syst Evol Microbiol. 68, 461-466.

658 [55] Ben Aissa, F., Postec, A., Erauso, G., Payri, C., Pelletier, B., Hamdi, M., Fardeau, M.L., Ollivier, B.
659 (2015) Characterization of *Alkaliphilus hydrothermalis* sp. nov., a novel alkaliphilic anaerobic
660 bacterium, isolated from a carbonaceous chimney of the Prony hydrothermal field, New Caledonia.
661 Extremophiles. 19, 183-188.

662 [56] Takai, K., Moser, D.P., Onstott, T.C., Spoelstra, N., Pfiffner, S.M., Dohnalkova, A., Fredrickson, J.K.
663 (2001) *Alkaliphilus transvaalensis* gen. nov., sp. nov., an extremely alkaliphilic bacterium isolated from
664 a deep South African gold mine. Int J Syst Evol Microbiol. 51, 1245-1256.

665 [57] Zakharyuk, A., Kozyreva, L., Ariskina, E., Troshina, O., Kopitsyn, D., Shcherbakova, V. (2017)
666 *Alkaliphilus namsaraevii* sp. nov., an alkaliphilic iron- and sulfur-reducing bacterium isolated from a
667 steppe soda lake. Int J Syst Evol Microbiol. 67, 1990-1995.

668 [58] Zhilina, T.N., Zavarzina, D.G., Kolganova, T.V., Lysneko, A.M., Tourova, T.P. (2009) *Alkaliphilus*
669 *peptidofragmentans* sp. nov., a new alkaliphilic bacterial soda lake isolate capable of peptide
670 fermentation and Fe(III) reduction. Mikrobiologiya. 78, 445-454.

671 [59] Cao, X., Liu, X., Dong, X. (2003) *Alkaliphilus crotonatoxidans* sp. nov., a strictly anaerobic,
672 crotonate-dismutating bacterium isolated from a methanogenic environment. Int J Syst Evol Microbiol.
673 53, 971-975.

674 [60] Mei, N., Postec, A., Monnin, C., Pelletier, B., Payri, C.E., Menez, B., Frouin, E., Ollivier, B., Erauso,
675 G., Quemeneur, M. (2016) Metagenomic and PCR-Based Diversity Surveys of [FeFe]-Hydrogenases
676 Combined with Isolation of Alkaliphilic Hydrogen-Producing Bacteria from the Serpentinite-Hosted
677 Prony Hydrothermal Field, New Caledonia. Front Microbiol. 7, 1301.

678 [61] Brazelton, W.J., Baross, J.A. (2009) Abundant transposases encoded by the metagenome of a
679 hydrothermal chimney biofilm. ISME J. 3, 1420-1424.

680 [62] Brazelton, W.J., Baross, J.A. (2010) Metagenomic comparison of two *Thiomicrospira* lineages
681 inhabiting contrasting deep-sea hydrothermal environments. PLoS ONE. 5, e13530.

682 [63] Frouin, E. (2018) "Taxonomic and functional exploration of the biosphere of serpentizing
683 hydrothermal systems by metagenomics" PhD Thesis. Aix-Marseille Univ.

684 [64] Wu, X.-Y., Shi, K.-L., Xu, X.-W., Wu, M., Oren, A., Zhu, X.-F. (2010) *Alkaliphilus halophilus* sp. nov.,
685 a strictly anaerobic and halophilic bacterium isolated from a saline lake, and emended description of
686 the genus *Alkaliphilus*. Int J Syst Evol Microbiol. 60, 2898-2902.

687 [65] Lecoeuve, A., Ménez, B., Cannat, M., Chavagnac, V., Gérard, E. (2020) Microbial ecology of the
688 newly discovered serpentinite-hosted Old City hydrothermal field (southwest Indian ridge). ISME J.

689 [66] Suzuki, S., Nealson, K.H., Ishii, S.i. (2018) Genomic and *in-situ* transcriptomic characterization of
690 the candidate phylum NPL-UPL2 from highly alkaline highly reducing serpentized groundwater. Front
691 Microbiol. 9.

692 [67] Lang, S.Q., Früh-Green, G.L., Bernasconi, S.M., Brazelton, W.J., Schrenk, M.O., McGonigle, J.M.
693 (2018) Deeply-sourced formate fuels sulfate reducers but not methanogens at Lost City hydrothermal
694 field. Sci Rep. 8, 755.

695 [68] McGonigle, J.M., Lang, S.Q., Brazelton, W.J. (2020) Genomic evidence for formate metabolism by
696 *Chloroflexi* as the key to unlocking deep carbon in lost city microbial ecosystems. Appl Environ
697 Microbiol. 86, e02583-02519.

698 [69] Maurizot, P., Vendé-Leclerc, M. (2009) Carte géologique de la Nouvelle-Calédonie au 1/500 000.
699 Direction de l'Industrie, des Mines et de l'Energie, Service de la Géologie de Nouvelle-Calédonie,
700 Bureau de Recherches Géologiques et Minières, Nouméa.

701 [70] Meier-Kolthoff, J.P., Göker, M. (2019) TYGS is an automated high-throughput platform for state-
702 of-the-art genome-based taxonomy. *Nat Commun.* 10, 2182.

703 [71] Ondov, B.D., Treangen, T.J., Melsted, P., Mallonee, A.B., Bergman, N.H., Koren, S., Phillippy, A.M.
704 (2016) Mash: fast genome and metagenome distance estimation using MinHash. *Genome Biol.* 17,
705 132.

706 [72] Lefort, V., Desper, R., Gascuel, O. (2015) FastME 2.0: A Comprehensive, Accurate, and Fast
707 Distance-Based Phylogeny Inference Program. *Mol Biol Evol.* 32, 2798-2800.

708 [73] Farris, J.S. (1972) Estimating Phylogenetic Trees from Distance Matrices. *Am Nat.* 106, 645-668.

709 [74] Suzuki, S., Ishii, S.i., Hoshino, T., Rietze, A., Tenney, A., Morrill, P.L., Inagaki, F., Kuenen, J.G.,
710 Nealson, K.H. (2017) Unusual metabolic diversity of hyperalkaliphilic microbial communities associated
711 with subterranean serpentinization at The Cedars. *ISME J.* 11, 2584-2598.

712 [75] Brazelton, W., Thornton, C., Hyer, A., Twing, K., Longino, A., Lang, S., Lilley, M., Früh-Green, G.,
713 Schrenk, M. (2017) Metagenomic identification of active methanogens and methanotrophs in
714 serpentinite springs of the Voltri Massif, Italy. *PeerJ* 5:e2945

715 [76] Twing, K.I., Brazelton, W.J., Kubo, M.D.Y., Hyer, A.J., Cardace, D., Hoehler, T.M., McCollom, T.M.,
716 Schrenk, M.O. (2017) Serpentinization-Influenced Groundwater Harbors Extremely Low Diversity
717 Microbial Communities Adapted to High pH. *Front Microbiol.* 8.

718 [77] Crespo-Medina, M., Twing, K.I., Sánchez-Murillo, R., Brazelton, W.J., McCollom, T.M., Schrenk,
719 M.O. (2017) Methane Dynamics in a Tropical Serpentinizing Environment: The Santa Elena Ophiolite,
720 Costa Rica. *Front Microbiol.* 8.

721 [78] Fortunato, C.S., Larson, B., Butterfield, D.A., Huber, J.A. (2018) Spatially distinct, temporally stable
722 microbial populations mediate biogeochemical cycling at and below the seafloor in hydrothermal vent
723 fluids. *Environ Microbiol.* 20, 769-784.

724 [79] Reveillaud, J., Reddington, E., McDermott, J., Algar, C., Meyer, J.L., Sylva, S., Seewald, J., German,
725 C.R., Huber, J.A. (2016) Subseafloor microbial communities in hydrogen-rich vent fluids from
726 hydrothermal systems along the Mid-Cayman Rise. *Environ Microbiol.* 18, 1970-1987.

727 [80] Dombrowski, N., Teske, A.P., Baker, B.J. (2018) Expansive microbial metabolic versatility and
728 biodiversity in dynamic Guaymas Basin hydrothermal sediments. *Nat Commun.* 9, 4999.

729 [81] Dong, X., Greening, C., Rattray, J.E., Chakraborty, A., Chuvochina, M., Mayumi, D., Dolfing, J., Li,
730 C., Brooks, J.M., Bernard, B.B., Groves, R.A., Lewis, I.A., Hubert, C.R.J. (2019) Metabolic potential of
731 uncultured bacteria and archaea associated with petroleum seepage in deep-sea sediments. *Nat*
732 *Commun.* 10, 1816.

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