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1 ***Crassaminicella indica* sp. nov., a novel thermophilic**
2 **anaerobic bacterium isolated from a deep-sea**
3 **hydrothermal vent**

4
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23 **Keywords:** *Crassaminicella indica*; thermophile; anaerobe; Central Indian Ridge

24 New tara: *Bacillota* and related organisms

25 Running title: *Crassaminicella indica* sp. nov.

26 The GenBank/EMBL/DDBJ accession number for the 16S rRNA sequence of strain 143-21^T is
27 OP271781. The complete genome sequence of 143-21^T has been deposited at GenBank/ENA/DDBJ
28 under the accession number CP078093.

30 ABSTRACT

31 A novel moderately thermophilic heterotrophic bacterium, designated strain 143-21^T, was isolated
32 from a deep-sea hydrothermal chimney sample collected on the Central Indian Ridge at a depth of
33 2,440 m. Phylogenetic analysis indicated that strain 143-21^T belongs to the genus *Crassaminicella*. It
34 was most closely related to *Crassaminicella thermophila* SY095^T (96.79 % 16S rRNA gene sequence
35 similarity) and *Crassaminicella profunda* Ra1766H^T (96.52 %). Genomic analysis showed that strain
36 143-21^T share 79.79–84.45 % average nucleotide identity (ANI) and 23.50–29.20 % digital DNA–
37 DNA hybridization (dDDH) with the species of the genus *Crassaminicella*, respectively. Cells were
38 rod-shaped, non-motile, Gram-positive-staining. Terminal endospores were observed in stationary-
39 phase cells when strain 143-21^T was grown on TRM medium. Strain 143-21^T was able to grow at 30–
40 60 °C (optimum 50 °C), pH6.5–8.5 (optimum 7.0), and in NaCl concentration of 1.0–7.0 % (w/v)
41 (optimum 2.0 %, w/v). Strain 143-21^T can utilize fructose, glucose, maltose, mannose, ribose, *N*-
42 acetyl-D-(+)-glucosamine and casamino acids, as well as amino acids including glutamate, lysine,
43 histidine, and cysteine. The main fermentation products from glucose were acetate (2.07 mM), H₂ and
44 CO₂. It did not reduce elemental sulfur, sulfate, thiosulfate, sulfite, fumarate, nitrate, nitrite and Fe
45 (III). The predominant cellular fatty acids were C_{14:0} (48.8 %), C_{16:0} (12.9 %), and summed Feature 3
46 contained C_{16:1} ω7c and/or C_{16:1} ω6c (10.2 %). The major polar lipids were diphosphatidylglycerol,
47 phosphatidylethanolamine, and phosphatidylglycerol, as well as two unidentified phospholipids, and
48 four unidentified aminolipid. No respiratory quinones were detected. Based on the phylogenetic
49 analysis and physiological characteristics, strain 143-21^T is considered to represent a novel species of
50 the genus *Crassaminicella*, for which the name *Crassaminicella indica* sp. nov. is proposed. The type
51 strain is strain 143-21^T (=DSM 114408^T = MCCC 1K06400^T).

52

53 Deep-sea hydrothermal vent represent a typical chemosynthetic ecosystem, which are fueled by
54 geothermal chemical energy (1, 2). The chemical-rich hydrothermal fluid erupted from the vent and
55 rapidly mixed with surrounding cold seawater, resulting in steep thermal and chemical gradients.
56 Chemoautotrophic microorganisms can exploit these chemical disequilibria to produce energy for
57 carbon fixation and support the vent food chain (3). Vent ecosystem also contains a high fraction of
58 heterotrophic microorganisms, such as *Alkaliphilus pronyensis* (4), *Caminiella sporogenes* (5),
59 *Clostridium tepidiprofundii* (6), *Paramaledivibacter caminithermalis* (7), *Serpentinicella alkaliphila*,
60 and *Wukongibacter baidiensis* (7). Interestingly, microbial communities inhabiting deep-sea
61 hydrothermal vent varies with the chimney from the cradle to the grave, heterotrophic
62 microorganisms would become dominant after the hydrothermal activity has ceased (8). In addition,
63 numerous heterotrophic groups have been isolated from hydrothermal sediments and exhibited
64 hydrolysis activity of hexadecane or polycyclic aromatic hydrocarbons (9). These versatile
65 heterotrophic groups play a vital role in the deep-sea biogeochemical cycles.

66 Species in the genus *Crassaminicella*, originated in the deep-sea environment, are heterotrophic and
67 was first proposed by R. Lakhali et al (10), which can ferment carbohydrates and organic acids. The
68 genus *Crassaminicella* belongs to the family *Clostridiaceae* of the order *Eubacteriales* of the class
69 *Clostridia* in the phylum *Bacillota*. At the time of writing, this genus includes only two valid
70 published species (<https://lpsn.dsmz.de/genus/crassaminicella>), *Crassaminicella profunda* Ra1766H^T
71 (10) isolated from the sediment of Guaymas Basin at a depth of 2,002 m, and *Crassaminicella*
72 *thermophila* SY095^T (11) isolated from the chimney sample of the Southwest Indian Ridge at a depth
73 of 2,730 m. Genome analysis has revealed their diverse metabolic characteristics and adaptive
74 strategies in the deep-sea environment (12, 13). Here, we report the isolation and characterization of a
75 novel species of the genus *Crassaminicella*, strain 143-21^T, originating from a deep-sea hydrothermal

76 vent in the Center Indian Ridge (CIR). The phenotypic and physiological features of novel species
77 will expand our knowledge about the genus *Crassaminicella*.

78

79 Isolation and Ecology

80 During the TS10-3 cruise of the research vessel ‘Tan Suo Yi Hao’ in February 2019, the active
81 chimney fragments were collected at a depth of 2,440 m from the *Kali* hydrothermal field (25.32°S,
82 70.04°E) of CIR, by using the manned submersible ‘Shen Hai Yong Shi’. The chimney surface was
83 covered by blind shrimps, few sea anemones attached to the rock near the root of the chimney. The
84 highest temperature of the hot vent fluid was 373 °C, which measured by a K-type thermocouple
85 probe inserted into the vent opening. The chimney fragments were obtained by the robotic arm of
86 manned submersible and placed in a closed biobox filled with sterile seawater. Once onboard, the
87 fragment was crushed under sterile conditions, and then stored anaerobically at 4 °C until culturing
88 attempts.

89 The stored chimney debris (about 0.5 g) were transferred into Hungate tubes containing 10 ml of
90 anaerobic Thermococcales Rich Medium (TRM) with N₂ as the headspace gas, and then incubated at
91 50 °C without shaking. After incubation over one week, the medium becomes cloudy indicating the
92 growing of microorganisms. To obtain pure cultures, the enrichment culture was spread anaerobically
93 on plates of TRM solidified with agar (2 %, w/v), and grow at 50 °C for one week. One colony was
94 picked for repeated plate streaking, the purity of the isolate was confirmed by microscopic
95 observations and by repeated sequencing of the 16S rRNA gene. The purified strain was designated
96 143-21^T and stored at –80 °C in TRM supplemented with 5 % (v/v) DMSO for long-term
97 preservation. The strain 143-21^T can also grow well in the JCM 909 medium composed of NaCl,
98 NH₄Cl, KH₂PO₄, K₂HPO₄, L-cysteine-HCl, MgCl₂·6H₂O, NaHCO₃, yeast extract and glucose etc. The
99 anaerobic JCM 909 medium was prepared as described by Li et al. (11). Further investigations were
100 carried out using JCM 909 medium unless otherwise stated.

101

102 16S RNA phylogeny

103 The complete sequence of the 16S rRNA gene (1,527 bp) of strain 143-21^T was retrieved from the
104 genomic sequence, and was then compared with available sequences in the NCBI GenBank
105 (nucleotide collection nr/nt database). 16S rRNA gene sequence analysis indicated that strain 143-21^T
106 belongs to the family *Clostridiaceae* of the phylum *Bacillota*. The closest phylogenetic relative is
107 *Crassaminicella thermophila* SY095^T (96.79 %), and *Crassaminicella profunda* Ra1766H^T (96.52 %),
108 followed by *Inediibacterium massiliense* Mt12^T (94.63 %), *Anaerophilus nitritogenes* MJB2^T
109 (94.45 %), *Geosporobacter ferrireducens* IRF9^T (94.00 %), *Marinisorobacter balticus* 59.4M^T
110 (93.58 %), *Wukongibacter baidiensis* DY30321^T (92.73 %), *Anaerosolibacter carboniphilus* IRF19^T
111 (92.29 %), *Salimesophilobacter vulgaris* Zn2^T (91.54 %). All the 16S rRNA gene sequences of the
112 closely related strains obtained from the GenBank database were aligned using sina aligner
113 (<https://www.arb-silva.de/aligner/>), phylogenetic analysis was performed by using the software
114 MEGA (version 11). A neighbor-joining phylogenetic tree of representative members was constructed
115 based on the 16S rRNA gene sequences. Bootstrap analysis was performed with 1000 replications.
116 Phylogenetic relationships showed that strain 143-21^T belonged to the genus *Crassaminicella* and
117 formed a clade with *C. thermophila* SY095^T and *C. profunda* Ra1766H^T (Fig. 1). The phylogenetic

118 topology of these *Crassaminicella* species was also confirmed using the minimum-evolution (Fig.
119 S1A) and maximum-likelihood methods (Fig. S1B).

120

121 **Genome Features**

122 For genomic DNA extraction, strain 143-21^T was grown on the JCM 909 medium at 50 °C. Cells
123 were harvested in late exponential-phase by centrifugation (4500 g, 20 min). Genomic DNA was
124 extracted using a MagAttract DNA Kit (Qiagen, USA) according to the manufacturer's instructions.
125 The whole-genome sequencing was carried out by Nextomics Biosciences Co., Ltd. (Wuhan, China)
126 by using the PromethION and MGI-SEQ 2000 systems. More than 2.5 Gb of clean data were
127 generated for the genome assembly. Briefly, strain 143-21^T genome contains a single circular
128 chromosome comprising 2,756,133 nucleotides with 31.10 % G+C content. A total of 2,427 protein-
129 coding sequences (CDS), 121 tRNA genes, and 33 rRNA genes including 5S, 16S, and 23S rRNAs
130 each containing 11 gene copies were identified (13). These 16S rRNA gene sequences show 99.94 %
131 similarity. The genome sequence of strain 143-21^T was deposited at DDBJ/ENA/GenBank with an
132 accession number of CP078093. To date, three *Crassaminicella* genomes have been reported. Among
133 them, strain 143-21^T and *C. thermophila* SY095^T are moderate thermophiles with optimal growth of
134 50 °C, while *C. profunda* Ra1766H^T is mesophilic with optimal growth of 30 °C. Comparative
135 genome analysis showed that the abundance of COG category in strain 143-21^T and *C. thermophila*
136 SY095^T, such as Nucleotide transport and metabolism (F), Coenzyme transport and metabolism (H),
137 Translation, ribosomal structure and biogenesis (J), and Mobilome: prophages, transposons (X), are
138 higher than the *C. profunda* Ra1766H^T. These differences may reflect the genome characteristics of
139 moderate thermophile, which will help them to survive in the deep-sea hydrothermal vent.

140 The phylogenetic tree based on the 120 concatenated bacterial marker proteins was constructed (14),
141 and the topology indicated that strain 143-21^T formed a distinct branch with *C. profunda* Ra1766H^T
142 and *C. thermophila* SY095^T (Fig. S2). Average nucleotide identity (ANI) was calculated by online
143 analysis sites EZBioCloud (<https://www.ezbiocloud.net/tools/ani>) (15). The DNA-DNA hybridization
144 (dDDH) between two genomes was calculated using GGDC 2.0 (<http://ggdc.dsmz.de/ggdc.php#>) (16).
145 The average amino acid identity (AAI) between strain 143-21^T and its closest phylogenetic relative
146 was determined using MiGA (17). Strain 143-21^T show 84.45 % and 79.79 % ANI value with *C.*
147 *profunda* Ra1766H^T and *C. thermophila* SY095^T respectively (Table S1), which are significantly
148 lower than the proposed threshold values of 95–96 % (18). The dDDH values between the genomes of
149 strain 143-21^T and two reference genomes were significantly lower than 70 % cutoff value previously
150 proposed for species delimitation (19), which are 29.20 % and 23.50 % for *C. profunda* Ra1766H^T
151 and *C. thermophila* SY095^T, respectively (Table S1). In addition, the AAI values between strain 143-
152 21^T and the related species (81.50 % and 66.78 % for *C. profunda* Ra1766H^T and *C. thermophila*
153 SY095^T, respectively) are also in the range of standard criteria (65 % < AAI < 95 %) for different
154 species of the same genus (20). Taken together, these results confirmed that strain 143-21^T is a
155 distinct species of the genus *Crassaminicella*.

156

157 **Physiology and Chemotaxonomy**

158 Strain 143-21^T was grown on the JCM 909 medium at 50 °C. The morphology of cells from late
159 exponential-phase was observed by phase-contrast microscopy (BX53 with a DP80 digital camera;
160 Olympus, Tokyo, Japan) and transmission electron microscopy (JEM-1230; JEOL Ltd., Tokyo,

161 Japan). Cells were straight rod-shaped, with a length of $7.1 \pm 1.5 \mu\text{m}$ and a width of $1.1 \pm 0.3 \mu\text{m}$. The
162 rods were non-motile without flagella (Fig. 2). Cells were stained using Gram Stain Kit (Beijing
163 Solarbio Science and Technology) and the results showed that cells were Gram-stain-positive.
164 Terminal endospores were observed in stationary-phase cells when the strain 143-21^T was grown on
165 TRM medium.

166 Physiological characterization of strain 143-21^T was carried out in the JCM 909 medium. 8 ml
167 anaerobic JCM 909 medium was dispensed into the serum bottle with a volume of 11 ml with N₂ as
168 the headspace gas. The prepared medium was inoculated with cells from late exponential-phase and
169 the final cell number is approximately 10^6 cells ml⁻¹. All experiments were carried out anaerobically
170 and all conditions were tested in triplicate unless stated otherwise. Growth was monitored by
171 measuring optical density at 600 nm as described by Dai et al. (21). Growth rates were calculated as
172 described by Li et al. (11) and shown in Fig. S3. The temperature range for growth was determined at
173 20–75 °C with 5 °C intervals. Strain 143-21^T grew over the range from 25 to 60 °C, the optimal
174 growth temperature is 50 °C. No growth was observed at 20 °C and 65 °C after 48 h of incubation.
175 The pH range for growth was tested at 50 °C from 5.0–9.0 at intervals of 0.5 pH units. The different
176 pH values were adjusted at room temperature (about 28 °C) with 5 M HCl or 5 M NaOH. For
177 different pH ranges, the JCM 909 medium was modified with following buffers (each at a
178 concentration of 10 mM): MES [2-(*N*-morpholino) ethanesulfonic acid] for pH 5.0–6.0; PIPES
179 (piperazine-*N*, *N*′-bis-2-ethane sulfonic acid) for pH 6.5–7.0; HEPES [4-(2-hydroxyethyl)-1-
180 piperazineethanesulfonic acid] for pH 7.5–8.5; and no buffer for pH 9.0. After autoclave, media with
181 various pH values were adjusted to required values with sterilized stock solutions of 5 M HCl or 5 M
182 NaOH. The pH growth range for strain 143-21^T was 6.5–8.5, and the optimal pH for growth was pH
183 7.0. No growth was observed at pH 6.0 or pH 9.0. To determine the NaCl tolerance, strain 143-21^T
184 was grown at 50 °C and pH 7.0. JCM 909 medium with gradients of 0–8 % NaCl (w/v) concentration
185 was prepared at 0.5 % intervals. Strain 143-21^T grew in NaCl concentrations of 1.0–7.0 % (w/v), with
186 optimum growth at 2.0 % (w/v).

187 For the analysis of the utilization of sole carbon sources, the basal 909 medium was prepared as
188 described by Lakhal *et al.* (10). The tested substrates were added to basal 909 medium with a final
189 concentration of 20 mM. Then, the prepared medium with various substrates were filtered through
190 sterilized 0.22 μm Millipore filters (Merck Millipore). These media were inoculated with cells from
191 late exponential-phase and the final cell number is approximately 10^6 cells ml⁻¹. The basal 909
192 medium and normal JCM 909 medium were also inoculated and set as the negative and positive
193 control, respectively. The following substrates were tested: fructose, glucose, maltose, mannose,
194 ribose, *N*-acetyl-D-(+)-glucosamine, cellobiose, galactose, glycerol, lactose, mannitol, raffinose,
195 rhamnose, sorbose, sucrose, xylose, tryptone, acetate, butyrate, formate, lactate, pyruvate, succinate,
196 ethanolamine, casamino acids, glutamate, lysine, histidine, cysteine, glutamine, methionine, serine,
197 alanine, arginine, asparagine, aspartate, glycine, isoleucine, leucine, phenylalanine, proline, tyrosine,
198 valine, tryptophan, and threonine. Strain 143-21^T was found to be able to utilize fructose, glucose,
199 maltose, mannose, ribose, and *N*-acetyl-D-(+)-glucosamine. Other single carbon sources were not
200 used under the tested condition. Strain 143-21^T was able to utilize casamino acids, glutamate, lysine,
201 histidine, and cysteine. Other amino acids including glutamine, methionine, serine, alanine, arginine,
202 asparagine, aspartate, glycine, isoleucine, leucine, phenylalanine, proline, tyrosine, valine, tryptophan,
203 and threonine were not utilized. The fermentation products of glucose were analyzed as described by
204 Li et al. (11). The main products of glucose fermentation were acetate (2.07 mM), H₂ and CO₂. The
205 ability of strain 143-21^T to use electron acceptors was tested by adding sulfur (1 %, w/v), sulfate (20
206 mM), thiosulfate (20 mM), sulfite (2 mM), fumarate (20 mM), nitrate (10 mM), nitrite (2 mM) and Fe
207 (III) (13 mM) to the JCM 909 medium, respectively. None were used as terminal electron acceptors.

208 The cellular fatty acid profiles of strain 143-21^T and reference strains were determined. Cells grown
209 on JCM 909 medium under optimal conditions were collected in late exponential-phase by
210 centrifugation (4500 g, 20 min). The optimal conditions is 50 °C, pH 7.0, NaCl 2.0 % (w/v) for strain
211 143-21^T, 50 °C, pH 7.0, NaCl 2.5 % (w/v) for *C. thermophila* SY095^T, and 30 °C, pH 7.0, NaCl 2.5 %
212 (w/v) for *C. profunda* Ra1776H^T. Fatty acids were prepared by the Marine Culture Collection of
213 China (MCCC) according to the instructions of the Microbial Identification System (MIDI), and were
214 then analyzed by gas chromatography (model 6850; Agilent Technologies) and identified by the
215 TSBA 6.0 database (22). The primary cellular fatty acids of strain 143-21^T were C_{14:0} (48.8 %), C_{16:0}
216 (12.9 %), and summed Feature 3 contained C_{16:1 ω7c} and/or C_{16:1 ω6c} (10.2 %). The dominant fatty
217 acid was C_{14:0} (48.8 %), which was same as *C. thermophila* SY095^T (C_{14:0}, 60.5 %) and *C. profunda*
218 Ra1776H^T (C_{14:0}, 44.9 %). However, strain 143-21^T contains higher proportion of C_{16:0} and C_{18:0} than
219 *C. thermophila* SY095^T and *C. profunda* Ra1776H^T (Table S2). The polar lipids were extracted using
220 a chloroform/methanol system and analyzed using one- & two-dimensional TLC. Strain 143-21^T show
221 a polar lipid composition of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), and
222 phosphatidylglycerol (PG), two unidentified phospholipids (PL1, PL2), and four unidentified
223 aminolipids (AL1, AL2, AL3, AL4) (Fig. S4). No respiratory quinones were detected which was
224 consistent with the results of the reference strain.

225 Overall, strain 143-21^T show a close phylogenetic relationship with *C. thermophila* SY095^T and *C.*
226 *profunda* Ra1776H^T from the genus *Crassaminicella*. However, strain 143-21^T could distinguished
227 with them by its optimal growth conditions (Table 1), physiological characterization (Table 1), cell
228 morphology, cellular fatty acids composition (Table S2), and genome relatedness. Therefore, we
229 suggested that strain 143-21^T (=DSM 114408^T = MCCC 1K06400^T) represents a novel species of the
230 genus *Crassaminicella*, for which the name *Crassaminicella indica* sp. nov. is proposed.

231 **Description of *Crassaminicella indica* sp. nov.**

232 *Crassaminicella indica* (in'di.ca. L. fem. adj. *indica*, pertaining to the Indian Ocean, where the strain
233 was isolated).

234 Cells are Gram-stain-positive, strictly anaerobic, non-motile, rod-shaped without flagella, 1.1 ± 0.3
235 μm in width and 7.1 ± 1.5 μm in length. Spores occur with terminal swelling in nutritional restriction.
236 Cell growth range is as followed: temperature 25–60 °C (optimum 50 °C), pH 6.5–8.5 (optimum 7.0),
237 NaCl 1.0–7.0 % (w/v) (optimum 2.0 %, w/v). Able to utilize fructose, glucose, maltose, mannose,
238 ribose, *N*-acetyl-D-(+)-glucosamine, casamino acids, glutamate, lysine, histidine, cysteine. Cellobiose,
239 galactose, glycerol, lactose, mannitol, raffinose, rhamnose, sorbose, sucrose, xylose, tryptone, acetate,
240 butyrate, formate, lactate, pyruvate, succinate, ethanolamine, glutamine, methionine, serine, alanine,
241 arginine, asparagine, aspartate, glycine, isoleucine, leucine, phenylalanine, proline, tyrosine, valine,
242 tryptophan, and threonine are not utilized. Terminal electron acceptors such as elemental sulfur,
243 sulfate, thiosulfate, sulfite, fumarate, nitrate, nitrite, and Fe (III) are not utilized. The major
244 fermentation products from glucose are acetate, H₂ and CO₂. The main cellular fatty acids consist of
245 C_{14:0} and C_{16:0}. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine,
246 phosphatidylglycerol, two unidentified phospholipids, and four unidentified aminolipid. No
247 respiratory quinones are detected.

248 The type strain, strain 143-21^T (=DSM 114408^T = MCCC 1K06400^T), was isolated from deep-sea
249 hydrothermal vent chimney at a depth of 2,440 m in the Kali hydrothermal field of the Central Indian
250 Ridge. The DNA G+C content of the type strain is 31.1 %. The GenBank/EMBL/DDBJ accession
251 number for the 16S rRNA sequence of strain 143-21^T is OP271781. The complete genome sequence
252 of 143-21^T has been deposited at GenBank/ENA/DDBJ under the accession number CP078093.

253

254

255 AUTHOR STATEMENTS

256

257 1.1 Authors and contributors

258 Xue-Gong Li designed the research outline. Ze-Xi Jiao and Xue-Gong Li drafted the manuscript. Ze-
259 Xi Jiao, Huan-Huan Zhang, Shi-Jie Bai, Jie Dai, Jin Lin, and Xiao-Qing Qi performed the
260 experiments. Xue-Gong Li, Wei-Jia Zhang and Long-Fei Wu collected the sample from the Central
261 Indian Ridge. Wei-Jia Zhang, Jun Xu, and Long-Fei Wu revised the manuscript. All authors read and
262 approved the final manuscript.

263 1.2 Conflicts of interest

264 The authors declare that there is no conflict of interest.

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274

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337

338 FIGURES AND TABLES

339 **Fig. 1.** Neighbor-joining phylogenetic tree based on 16S rRNA sequences of strain 143-21^T and its
340 relatives. Bootstrap values above 50 % based on 1,000 replicates are shown at nodes. Filled circles
341 indicate nodes also recovered with minimum evolution and maximum-likelihood methods. The
342 respective GenBank accession numbers are given in parentheses. Bar, 2 nucleotide substitutions per
343 1,000 nucleotide positions.

344 **Fig. 2.** Transmission-electron microscopy image of a cell of strain 143-21^T. Bar: 1 μ m.

345

346

347 **Table 1.** Differential phenotypic characteristics of strain 143-21^T and its closely related species

348 Strains: 1, *Crassaminicella indica* 143-21^T; 2, *Crassaminicella thermophila* SY095^T; 3,
349 *Crassaminicella profunda* Ra1766H^T. +, Positive; –, negative.

350

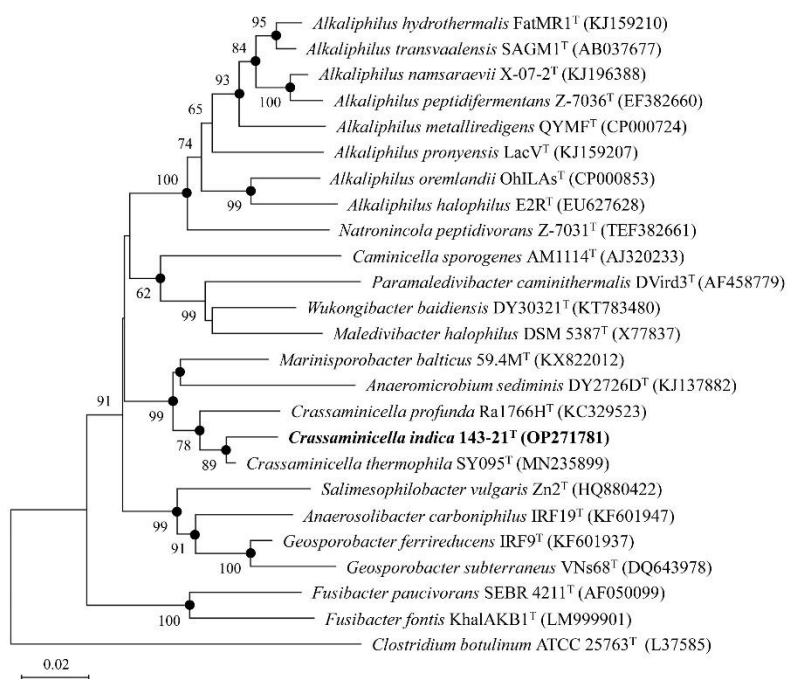
Characteristics	1	2	3
Geographical origin	Central Indian Ridge	Southwest Indian Ridge	Guaymas basin
Depth (m)	2,440	2,730	2,002
Motility	-	+	+
Size (μ m)	1.1 \pm 0.3 \times 7.1 \pm 1.5	0.7 \pm 0.1 \times 6.1 \pm 1.0	0.5–1.0 \times 6.0–10.0
Presence of spores	+	+	+
Gram-stain	+	+	+
Growth conditions*#			
Temperature range ($^{\circ}$ C)	25–60 (50)	45–60 (50–55)	20–40 (30)
pH range	6.5–8.5 (7.0)	6.0–7.5 (7.0)	6.0–8.0 (7.0)
NaCl (% w/v)	1.0–7.0 (2.0)	1.0–4.5 (2.5)	0.5–4.0 (2.5)
Substrates used#			
Fructose	+	+	-
Maltose	+	+	-

Mannose	+	-	+
Ribose	+	-	-
Acetate	-	-	+
Pyruvate	-	-	+
<i>N</i> -acetyl-D-(+)-glucosamine	+	+	-
DNA G+C content (%)	31.1	30.8	31.5

351 * Optimum values in parenthesis.

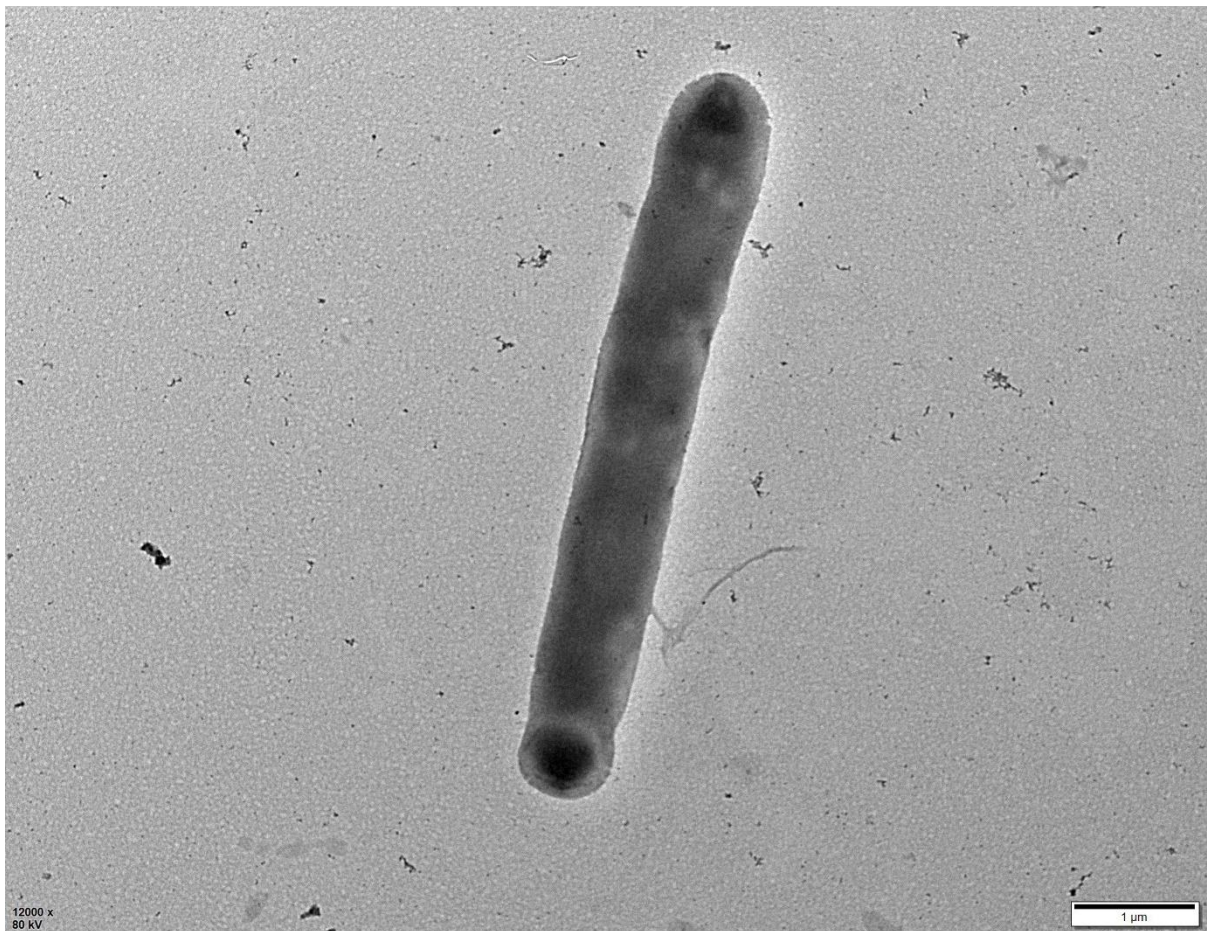
352 # Data are from this study.

353 Fig 1



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355



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358

359 **Supplementary Material Files**

360 ***Crassaminicella indica* sp. nov., a novel thermophilic**
361 **anaerobic bacterium isolated from a deep-sea**
362 **hydrothermal vent**

363

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365 Zhang^{1,3,4,5}, Xiao-Qing Qi^{1,3,4}, Long-Fei Wu^{3,9}

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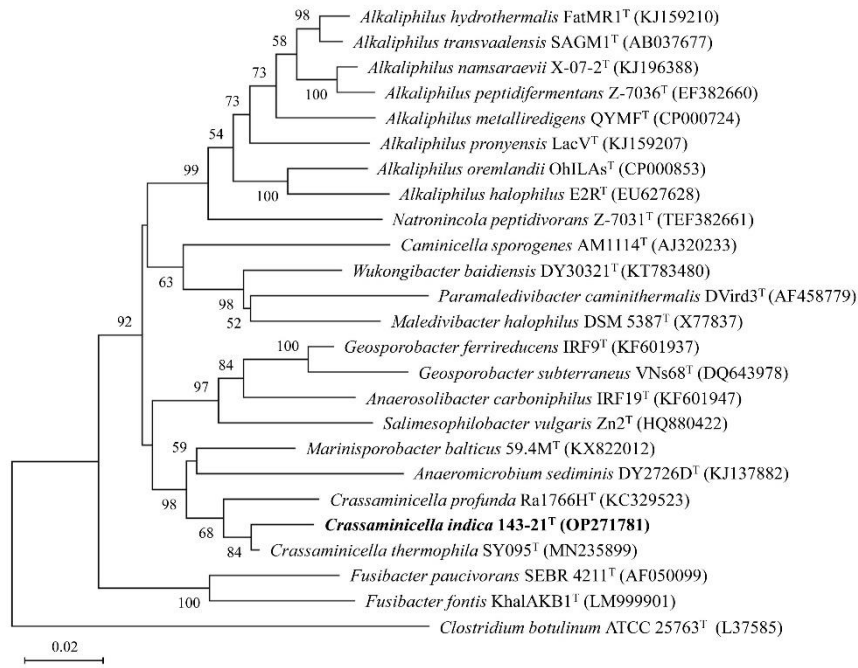
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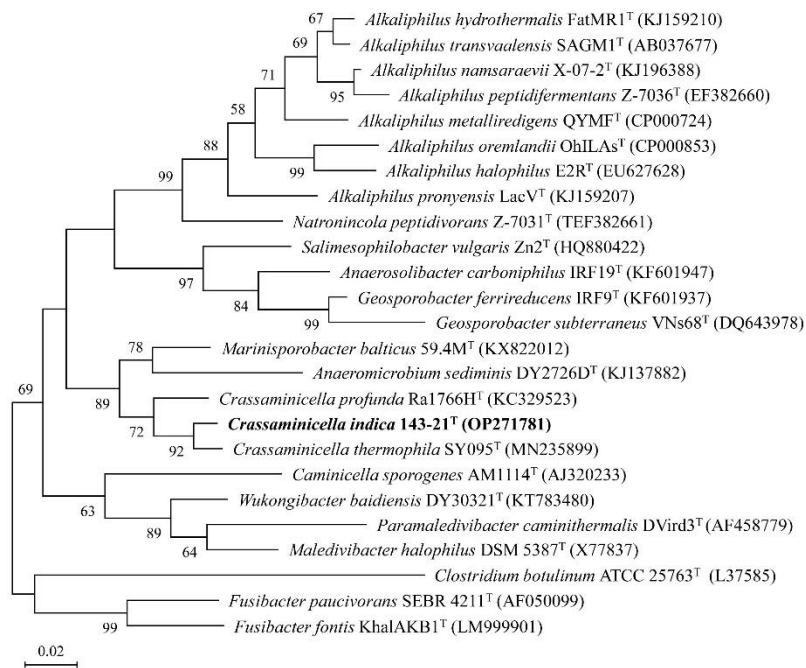
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A



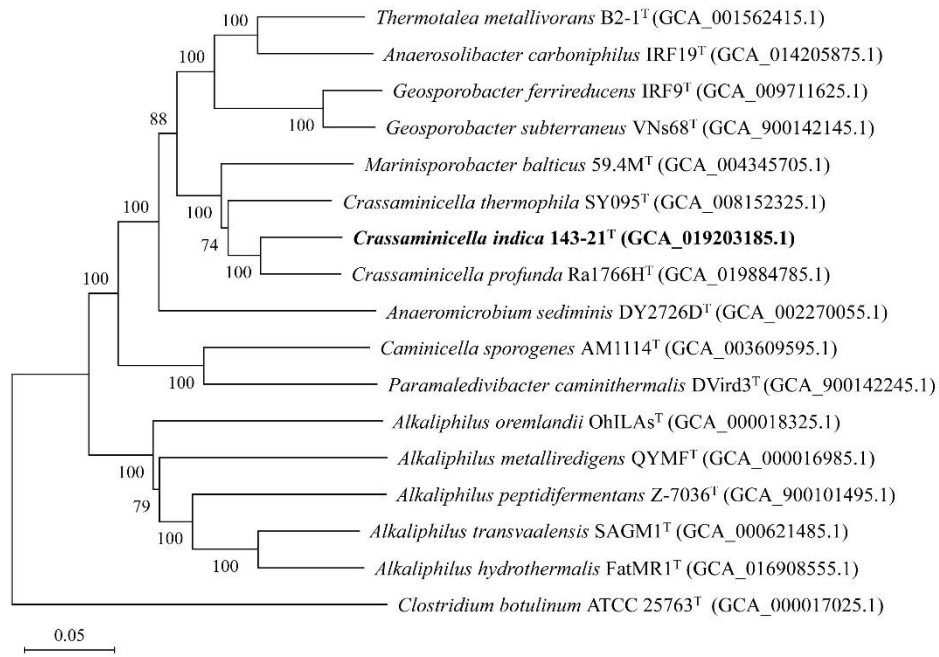
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B

386 Fig. S1. Minimum evolution (A) and maximum likelihood (B) phylogenetic trees based on 16S rRNA
 387 sequences of strain 143-21^T and its closest relatives. Bootstrap values above 50 % based on 1,000
 388 replicates are shown at nodes. The respective GenBank accession numbers are given in parentheses.
 389 Bar, 2 nucleotide substitutions per 1,000 nucleotide positions.

390

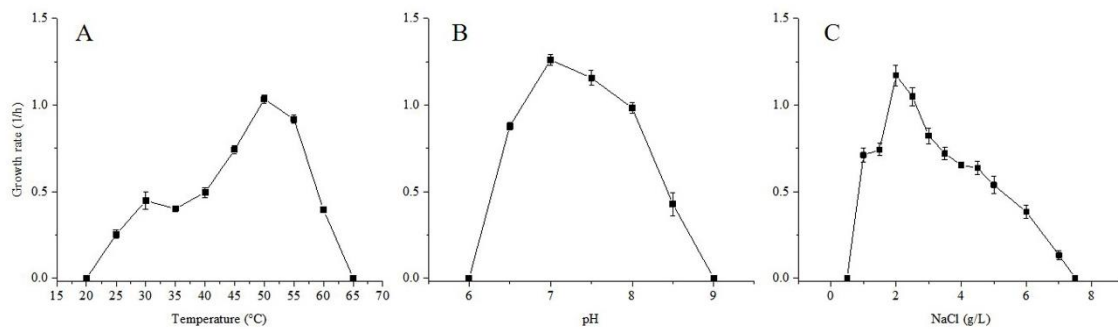
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392

393 Fig. S2. Neighbor-joining phylogenetic tree of strain 143-21^T and its closest relatives based on the
 394 concatenated 120 bacterial marker proteins using the MEGA version 11. The evolutionary distances
 395 were computed using the p-distance method and are in the units of the number of amino acid
 396 differences per site. The respective GenBank accession numbers are given in parentheses. Bootstrap
 397 values above 50 % based on 1,000 replicates are shown at nodes. Bar, number of amino acid
 398 differences per site.

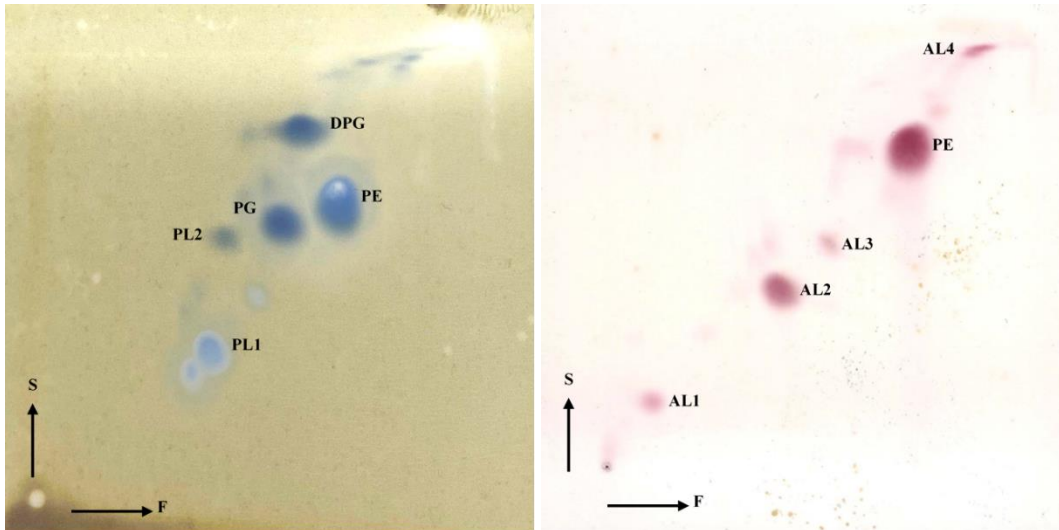
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400

401 Fig. S3. Effects of temperature (A), pH (B) and NaCl concentration (C) on the growth of strain 143-21^T. Each
 402 value is the mean of three replicates, mean values \pm SD, n=3.

403



404

405 Fig. S4. Polar lipids composition of strain 143-21^T. PL: Phospholipid, PG: Phosphatidylglycerol,
406 DPG: Diphosphatidylglycerol, PE: phosphatidylethanolamine, AL, aminolipid, F-first dimension of
407 TLC; S- second dimension of TLC.

408

409 **Supplementary Table S1.** Whole genome-based comparisons between strain 143-21^T and closely
 410 relatives.

Strains	DDH (%)	ANI (%)	AAI (%)
<i>Crassaminicella profunda</i> Ra1766H ^T	29.20	84.85	81.50
<i>Crassaminicella thermophila</i> SY095 ^T	23.50	79.79	66.78

411

412 **Supplementary Table S2.** Fatty acid composition of strain 143-21^T and its closely related strains.
 413 Strain: 1, 143-21^T; 2, *Crassaminicella thermophila* SY095^T; 3, *Crassaminicella profunda* Ra1766H^T.
 414 Values are percentages of total fatty acids. Fatty acids that represented <1 % in all strains were
 415 omitted. TR, Traces (<1 %).

Fatty acid*	Composition (%) in:		
	1	2	3
C _{12:0}	TR	1.0	TR
C _{14:0}	48.8	60.5	44.9
C _{16:1} ω9c	3.4	3.4	9.0
C _{16:0}	12.9	7.6	7.0
C _{18:0}	3.8	TR	TR
Summed Feature 1	2.7	5.6	1.9
Summed Feature 3	10.2	14.0	29.6
Summed Feature 4	9.9	3.6	3.7
Summed Feature 8	3.25	TR	TR

416 *Summed Features are fatty acids that cannot be resolved reliably from another fatty acid using the
 417 chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature
 418 with a single percentage of the total. Summed Feature 1 contained iso-C_{15:1} H and/or C_{13:0} 3-OH;
 419 Summed Feature 3 contained C_{16:1} ω7c and/or C_{16:1} ω6c; Summed Feature 4 contained iso-C_{17:1} I
 420 and/or anteiso-C_{17:1} B; Summed Feature 8 contained C_{18:1} ω7c and/or C_{18:1} ω6c.

421