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***Salimicrobium salexigens* sp. nov., a moderately halophilic bacterium from salted hides**

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Note: Nucleotide sequence data for the 16S rRNA gene are available in the GenBank/EMBL/DDBL databases under the accession numbers: FR714935 (strain 29CMI<sup>T</sup>) and FR714936 (strain 53CMI).

26 **Abstract**

27 Two Gram-positive, moderately halophilic bacteria, designated strains 29CMI<sup>T</sup>  
28 and 53CMI, were isolated from salted hides. Both strains were non-motile, strictly  
29 aerobic cocci, growing in the presence of 3-25 % (w/v) NaCl (optimal growth at 7.5-  
30 12.5 % [w/v] NaCl), between pH 5.0 and 10.0 (optimal growth at pH 7.5) and at  
31 temperatures between 15 and 40 °C (optimal growth at 37 °C). Phylogenetic analysis  
32 based on 16S rRNA gene sequence comparison showed that both strains showed a  
33 similarity of 98.7 % and were closely related to species of the genus *Salimicrobium*,  
34 within the phylum *Firmicutes*. Strains 29CMI<sup>T</sup> and 53CMI exhibited 16S rRNA gene  
35 sequence similarity values of 97.9 % to 97.6 % with *Salimicrobium album* DSM  
36 20748<sup>T</sup>, *Salimicrobium halophilum* DSM 4771<sup>T</sup>, *Salimicrobium flavidum* ISL-25<sup>T</sup> and  
37 *Salimicrobium luteum* BY-5<sup>T</sup>. The DNA G+C content was 50.7 mol% and 51.5 mol%  
38 for strains 29CMI<sup>T</sup> and 53CMI, respectively. The DNA-DNA hybridization between  
39 both strains was 98 %, whereas the values between strain 29CMI<sup>T</sup> and the species  
40 *Salimicrobium album* CCM 3517<sup>T</sup>, *Salimicrobium luteum* BY-5<sup>T</sup>, *Salimicrobium*  
41 *flavidum* ISL-25<sup>T</sup> and *Salimicrobium halophilum* CCM 4074<sup>T</sup> were 45 %, 28 %, 15 %  
42 and 10 %, respectively, showing unequivocally that strains 29CMI<sup>T</sup> and 53CMI  
43 constitute a new genospecies. The major cellular fatty acids were anteiso-C<sub>15:0</sub>, anteiso-  
44 C<sub>17:0</sub>, iso-C<sub>15:0</sub> and iso-C<sub>14:0</sub>. The main respiratory isoprenoid quinone was MK-7,  
45 although small amounts of MK-6 were also found. The polar lipids of the type strain  
46 consist of diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid  
47 and one glycolipid. The peptidoglycan type is A1γ, with *meso*-diaminopimelic acid as  
48 the diagnostic diamino acid. On the basis of the phylogenetic analysis, and phenotypic,  
49 genotypic and chemotaxonomic characteristics, we propose strains 29CMI<sup>T</sup> and 53CMI

50 as a novel species of the genus *Salimicrobium*, with the name *Salimicrobium salexigens*  
51 sp. nov. The type strain is 29CMI<sup>T</sup> (= CECT 7568<sup>T</sup> =JCM 16414<sup>T</sup> = LMG 25386<sup>T</sup>).

52

53 **Keywords:** Moderately halophilic bacteria, *Salimicrobium*, *Salimicrobium salexigens*,  
54 *Firmicutes*, salted hides.

55 **Scope:** Systematics

56

## 57 **Introduction**

58 The genus *Salimicrobium* was proposed by Yoon et al. [32] in order to transfer  
59 two previously described species, *Marinococcus albus* [9] and *Bacillus halophilus* [28],  
60 as *Salimicrobium album* and *Salimicrobium halophilum*, respectively, and to  
61 accommodate a new species, *Salimicrobium luteum* [32]. The genus *Salimicrobium*  
62 includes Gram-positive, strictly aerobic rods and cocci which are within the *Firmicutes*;  
63 they are catalase- and oxidase positive, moderately halophilic bacteria which require  
64 NaCl for growing. The predominant menaquinone is MK-7 and its cells wall  
65 peptidoglycan contains *meso*-diaminopimelic acid as the diagnostic diaminoacid. The  
66 DNA G+C content is 44.9-51.5 mol%. The type species of this genus is *Salimicrobium*  
67 *album*, which was originally isolated from a solar saltern in Spain [31]. The other two  
68 species, *S. halophilum* and *S. luteum* were isolated from marine solar salterns in Spain  
69 and Korea, respectively [28,32]. More recently, another species has been described  
70 within this genus: *Salimicrobium flavidum* isolated from a marine solar saltern of the  
71 Yellow Sea in Korea [33].

72           Recent studies focused on the determination of the microbial diversity of salted  
73 hides permitted us to isolate a new species of the genus *Thalassobacillus*,  
74 *Thalassobacillus pellis* [23]. The aim of this study was to determine the taxonomic  
75 position of two new isolates, strains 29CMI<sup>T</sup> and 53CMI, using a polyphasic approach.  
76 Our results show that these isolates represent a novel species of the genus  
77 *Salimicrobium*, for which we propose the new designation *Salimicrobium salexigens* sp.  
78 nov.

79

## 80 **Materials and methods**

### 81 *Isolation and bacterial strains*

82           Strains 29CMI<sup>T</sup> and 53CMI were isolated from salted hides obtained from  
83 Australia on SW15 medium which contained a mixture of 15% salts [30] after  
84 incubation at 37 °C for 7 days. For routine growth the strains were cultivated in SW10  
85 medium with 10 % (w/v) total salts (8.1 % NaCl, 0.7 % MgCl<sub>2</sub>, 0.96 % MgSO<sub>4</sub>, 0.036  
86 % CaCl<sub>2</sub>, 0.2 % KCl, 0.006 % NaHCO<sub>3</sub>, 0.0026 % NaBr, 0.5 % yeast extract [Difco])  
87 [30]. The pH was adjusted to 7.5 with 1 M KOH. When necessary, solid media were  
88 prepared by adding 2.0 % (w/v) Bacto-agar (Difco). These cultures were maintained at -  
89 80 °C in SW10 medium containing 50 % (v/v) glycerol. The type strains of  
90 *Salimicrobium album* CCM 3517<sup>T</sup>, *Salimicrobium halophilum* CCM 4074<sup>T</sup>,  
91 *Salimicrobium luteum* BY-5<sup>T</sup> and *Salimicrobium flavidum* ISL-25<sup>T</sup> were used for  
92 comparative purposes. They were cultivated under the same conditions than strains  
93 29CMI<sup>T</sup> and 53CMI.

### 94 *Phenotypic characterization*

95           The proposed minimal standards for describing new taxa of aerobic, endospore-  
96 forming bacteria as recommended by Logan et al. [12] were followed. For the  
97 determination of cellular morphology and motility, a culture from liquid 10 % HM  
98 medium was examined by light microscopy under a phase-contrast microscope. The  
99 morphology of colonies, their size and pigmentation were observed on the 10 % HM  
100 solid medium after 48 h of incubation at 37 °C. The composition of the 10 % HM  
101 medium is (w/v): 8.1 % NaCl, 0.7 % MgCl<sub>2</sub>, 0.96 % MgSO<sub>4</sub>, 0.036 % CaCl<sub>2</sub>, 0.2 %  
102 KCl, 0.006 % NaHCO<sub>3</sub>, 0.0026 % NaBr, 0.5 % proteose peptone (Difco), 1.0 % yeast  
103 extract (Difco), 0.1 % glucose and 1.5 % agar [30]. Optimal conditions for growth were  
104 determined by growing the strains in SW medium at 0, 0.5, 3.0, 5, 7.5, 10, 12.5, 15, 20,  
105 25 and 30 % (w/v) NaCl, and at temperatures of 4, 15, 20, 28, 30, 37, 40 and 45 °C,  
106 respectively. The pH range for the isolates was tested in SW10 medium adjusted to the  
107 following pH values: 4.0, 5.0, 6.0, 7.0, 7.5, 8.0, 9.0 and 10.0 with the addition of the  
108 appropriate buffering capacity to each medium [22]. All biochemical tests were carried  
109 out at 10 % NaCl and 37 °C, unless it is stated otherwise. Growth under anaerobic  
110 conditions was determined by incubating strains in an anaerobic chamber in SW10  
111 medium. Catalase activity was determined by adding a 1 % (w/v) H<sub>2</sub>O<sub>2</sub> solution to  
112 colonies on SW10 agar medium. Oxidase test was performed using the Dry Slide Assay  
113 (Difco). Hydrolysis of aesculin, casein, DNA, gelatin, starch, Tween 80, pullulan and  
114 xylan, Voges-Proskauer and methyl red tests, production of indole, arginine, lysine and  
115 ornithine decarboxylases, phenylalanine deaminase, phosphatase, urease and nitrate  
116 reduction were determined as described by Cowan & Steel [4] with the addition of a  
117 10% total salts to the medium [19,30]. Citrate utilization was determined on Simmon's  
118 Citrate medium supplemented with SW10. Acid production from carbohydrates was  
119 determined using phenol red base supplemented with 1 % of the carbohydrate and

120 SW10 medium [30]. For determining the range of substrates used as carbon and energy  
121 sources or as carbon, nitrogen and energy sources, the classical medium of Koser [11]  
122 as modified by Ventosa et al. [30] was used: 75 g NaCl l<sup>-1</sup>, 2 g KCl l<sup>-1</sup>, 0.2 g  
123 MgSO<sub>4</sub>·7H<sub>2</sub>O l<sup>-1</sup>, 1 g KNO<sub>3</sub> l<sup>-1</sup>, 1 g (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub> l<sup>-1</sup>, 0.5 g KH<sub>2</sub>PO<sub>4</sub> l<sup>-1</sup> and 0.05 g yeast  
124 extract (Difco) l<sup>-1</sup>. Substrates were added as filter-sterilized solutions to give a final  
125 concentration of 1 g l<sup>-1</sup>, except for carbohydrates, which were used at 2 g l<sup>-1</sup>. When the  
126 substrate was an amino acid, it was tested as carbon, nitrogen and energy source, and  
127 the basal medium was therefore prepared without KNO<sub>3</sub> and (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>.

### 128 *Phylogenetic analysis*

129 Genomic DNA from strains 29CMI<sup>T</sup> and 53CMI was prepared using the method  
130 described by Marmur [14]. Their 16S rRNA gene was amplified by PCR with the  
131 forward primer 16F27 and the reverse primer 16R1488 [16]. Direct sequence  
132 determination of the PCR-amplified DNA was carried out using an automated DNA  
133 sequencer (model ABI 3130XL; Applied Biosystems). The 16S rRNA gene sequence  
134 analysis was performed with the ARB software package [13]. The 16S rRNA gene  
135 sequences were aligned with the published sequences of the closely related bacteria and  
136 the alignment was confirmed and checked against both primary and secondary  
137 structures of the 16S rRNA molecule using the alignment tool of the ARB software  
138 package. Phylogenetic trees were constructed using three different methods: maximum-  
139 likelihood [6], maximum-parsimony [8] and neighbour-joining [21], algorithms  
140 integrated in the ARB software for phylogenetic inference. Bootstrap test [7] was  
141 performed by calculating 1000 replicate trees in order to assess the robustness of the  
142 topology. The 16S rRNA gene sequences used for phylogenetic comparisons were

143 obtained from the GenBank database and their strain designations and accession  
144 numbers are shown in Figure 1.

#### 145 *Chemotaxonomic analysis*

146 Fatty acids analysis was performed using the MIDI system (Microbial  
147 Identification System). Cells were cultured on Marine agar (MA) medium (Difco) at 37  
148 °C for 24 h. The extraction and analysis of fatty acids were performed according to the  
149 recommendations of the MIDI system. This analysis was carried out by the  
150 Identification, Characterization and Molecular Typing Service of the BCCM/LMG  
151 Bacteria Collection (Gent, Belgium). The peptidoglycan structure of strains 29CMI<sup>T</sup>  
152 and 53CMI was determined by Dr. Peter Schumann from the Identification Service of  
153 the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen),  
154 Braunschweig, Germany. The determination was carried out as described by Schleifer  
155 [24] and Schleifer and Kandler [25] by thin-layer chromatography on cellulose plates  
156 using the solvent system of Rhuland et al. [20].

157 The analysis of respiratory quinones and polar lipids of strain 29CMI<sup>T</sup> was  
158 carried out by Dr. Brian Tindall, also from the Identification Service of the DSMZ. The  
159 quinones were determined according to the method of Collins et al. [3]. The polar lipids  
160 were analysed using the method of Minnikin et al. [17] and Collins and Jones [2].

#### 161 *Determination of the DNA G+C content and DNA-DNA hybridization*

162 The G+C content of the genomic DNA was determined from the midpoint value  
163 of the thermal denaturation profile [15] using the equation of Owen & Hill [18]. DNA-  
164 DNA hybridization studies were performed by the competition procedure of the  
165 membrane filter method [10]. The hybridization temperature was 51.2 °C, which is

166 within the limit of validity for the filter method [5] and the percentage of hybridization  
167 was calculated according to Johnson [10]. The experiments were performed in triplicate.

168

## 169 **Results and discussion**

170 Strains 29CMI<sup>T</sup> and 53CMI were Gram-positive, non-motile and strictly aerobic  
171 cocci. They were able to grow in media containing 3-25 % (w/v) NaCl and optimally in  
172 media containing 7.5-12.5 % and 10 % (w/v) NaCl, respectively. Both strains were  
173 unable to grow in the absence of NaCl. On the basis of the NaCl requirements these  
174 bacteria can be considered as moderately halophilic microorganisms [29]. Their optimal  
175 temperature and pH were 37 °C and pH 7.5. Both strains showed very similar  
176 phenotypic features and their characteristics are detailed in the new species description  
177 and Table 1. They showed some differences with respect to the production of acid from  
178 some carbohydrates and the utilization of some compounds, as reported in the new  
179 species description.

180 The almost-complete 16S rRNA gene sequences of strains 29CMI<sup>T</sup> (1481 bp)  
181 and 53CMI (1438 bp) were obtained and used for initial BLAST searches in GenBank  
182 and phylogenetic analysis. The identification of phylogenetic neighbours and  
183 calculation of pairwise 16S rRNA gene sequence similarity were achieved using the  
184 ARB software package [13] and the EzTaxon server (<http://www.eztaxon.org/>; [1]). The  
185 phylogenetic analysis, based on the maximum-parsimony algorithm, revealed that  
186 strains 29CMI<sup>T</sup> and 53CMI formed a monophyletic group with the species of the genus  
187 *Salimicrobium* (Fig. 1). Neighbour-joining and maximum-likelihood methods resulted  
188 in highly similar tree topologies (Supplementary Figures S1 and S2). The 16S rRNA



189 gene sequence similarity between strains 29CMI<sup>T</sup> and 53CMI was 98.7%. The closest  
190 phylogenetic similarity of strains 29CMI<sup>T</sup> and 53CMI with other bacterial species was  
191 with the type strains of *Salimicrobium album* DSM 20748<sup>T</sup> (97.8 % and 97.8 % 16S  
192 rRNA sequence similarity, respectively), *Salimicrobium halophilum* DSM 4771<sup>T</sup> (97.8  
193 % and 97.7 %), *Salimicrobium flavidum* ISL-25<sup>T</sup> (97.7 % and 97.9 %) and  
194 *Salimicrobium luteum* BY-5<sup>T</sup> (97.6 % and 97.7 %). Lower 16S rRNA sequence  
195 similarity was found with respect to species of *Halobacillus* (equal or lower than 94.2  
196 %) and *Thalassobacillus* (equal or lower than 92.7 %). In order to determine if the new  
197 isolates constituted a new species of the genus *Salimicrobium*, we carried out DNA-  
198 DNA hybridization studies between the two strains and between strain 29CMI<sup>T</sup>, which  
199 was selected as the type strain of the new taxon, and the type strains of the species of  
200 the genus *Salimicrobium*.

201 The percentage of DNA-DNA hybridization (DDH) between strain 29CMI<sup>T</sup> and  
202 strain 53CMI was 98%; which is higher than 70%, currently accepted as the cut-off  
203 value for species delineation [26,27]. The DDH relatedness of strain 29CMI<sup>T</sup> and the  
204 type strains of the species *Salimicrobium album* CCM 3517<sup>T</sup>, *Salimicrobium luteum*  
205 BY-5<sup>T</sup>, *Salimicrobium flavidum* ISL-25<sup>T</sup> and *Salimicrobium halophilum* CCM 4074<sup>T</sup>  
206 were 45 %, 28 %, 15 % and 10 %, respectively. These levels of DNA-DNA  
207 hybridization are low enough to consider the new strains as a genotypically distinct  
208 species within the genus *Salimicrobium* [26,27].

209 The G+C content of the DNA for strains 29CMI<sup>T</sup> and 53CMI was 50.7 and 51.5  
210 mol %, respectively. These values are within the range (44.9-51.5 mol %) for other  
211 species of the genus *Salimicrobium* (Table 1).

212 The cellular fatty acid profile of strains 29CMI<sup>T</sup> and 53CMI was characterized  
213 by the fatty acids anteiso-C<sub>15:0</sub>, anteiso-C<sub>17:0</sub>, iso-C<sub>15:0</sub> and iso-C<sub>14:0</sub> as the major fatty  
214 acids (Table 2). The fatty acid composition is similar to those found in the other species  
215 of *Salimicrobium*, except for the absence of iso-C<sub>14:0</sub> for *Salimicrobium album* and a  
216 higher percentage of iso-C<sub>17:0</sub> for species *Salimicrobium halophilum* and *Salimicrobium*  
217 *luteum* (Table 2). The cell-wall peptidoglycan analysis showed the presence of *meso*-  
218 diaminopimelic acid as diagnostic diamino acid of the peptidoglycan in strains 29CMI<sup>T</sup>  
219 and 53CMI, indicating that they have the peptidoglycan type A1 $\gamma$ , in accordance with  
220 the type reported for other species of the genus *Salimicrobium* [9,28,32,33].

221 Strain 29CMI<sup>T</sup> contained MK-7 as the predominant isoprenoid quinone (97%)  
222 and minor amounts of MK-6 (3 %). The major quinone reported for the species of the  
223 genus *Salimicrobium* is also MK-7 [9,28,32,33]. The polar lipids of strain 29CMI<sup>T</sup>  
224 consist of diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid  
225 and one glycolipid.

226 The characteristics that differentiate strains 29CMI<sup>T</sup> and 53CMI from the  
227 related *Salimicrobium* species are summarized in Table 1. On the basis of the  
228 phylogenetic, genotypic, chemotaxonomic and phenotypic data, we propose that both  
229 strains be classified in a novel species of the genus *Salimicrobium*, as *Salimicrobium*  
230 *salexigens* sp. nov.

231

### 232 **Description of *Salimicrobium salexigens* sp. nov.**

233 *Salimicrobium salexigens* (sal.e.xi'gens. L. n. *salt*, salt; L. v. *exigo*, to demand;  
234 M. L. part. adj. *salexigens*, salt demanding).

235 Cells are Gram-positive, non-motile, cocci, 1.0-2.0  $\mu\text{m}$  in size, that occur singly,  
236 in pairs or tetrads. Colonies are circular, entire, smooth, convex, yellow pigmented and  
237 1-2 mm in diameter on 10 % HM agar medium after 48 h incubation at 37 °C. Strictly  
238 aerobic. Moderately halophilic, growing at 3-25 % (w/v) NaCl; with optimal growth at  
239 7.5-12.5 % (w/v) NaCl. No growth occurs in the absence of NaCl. Grows at 15-40 °C;  
240 showing optimal growth at 37 °C, and at pH values on the range 5.0-10.0; with optimal  
241 growth at pH 7.5. Catalase and oxidase positive. DNA is hydrolysed but gelatin, casein,  
242 pullulan, starch, aesculin, Tween 80 and xylan are not hydrolysed. Nitrate is reduced to  
243 nitrite. Nitrite not reduced. Acid is produced from D-glucose, D-fructose, maltose,  
244 sucrose and D- trehalose but not from D-amydaline, D-arabinose, arbutine, cellobiose,  
245 L-citruline, dulcitol, DL-ethionine, *myo*-inositol, inuline, lactose, melezitose, melibiose,  
246 D-ribose, raffinose, sorbitol, xylitol or D-xylose. Indole or H<sub>2</sub>S are not produced.  
247 Phosphatase is positive. Methyl red, Voges-Proskauer, Simmons' citrate, urease,  
248 arginine, lysine and ornithine decarboxylases and phenylalanine deaminase tests are  
249 negative. The following compounds are utilized as sole sources of carbon and energy:  
250 D-galactose, D-glucose, ribose, D-mannitol, D-sorbitol and acetate. The following  
251 compounds are not utilized as sole sources of carbon and energy: D-arabinose, D-  
252 cellobiose, D-fructose, D-fucose, lactose, aesculin, D-melizitose, salicin, starch,  
253 butanol, dulcitol, *myo*-inositol, methanol, benzoate, citrate, formate, fumarate,  
254 hippurate, malate, propionate, succinate, tartrate and valerate. The following compounds  
255 are not utilized as sole sources of carbon, nitrogen and energy: L-arginine, aspartate, L-  
256 cysteine, L-threonine, tryptophan and valine. The predominant cellular fatty acids are  
257 anteiso-C<sub>15:0</sub>, anteiso-C<sub>17:0</sub>, iso-C<sub>15:0</sub> and iso-C<sub>14:0</sub>. The respiratory isoprenoid quinones  
258 are MK-7 (97%) and MK-6 (3%). The polar lipids of the type strain consist of  
259 diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid and one

260 glycolipid. The peptidoglycan type is A1 $\gamma$ , with *meso*-diaminopimelic acid as the  
261 diagnostic diamino acid. The DNA G+C content is 50.7-51.5 mol% ( $T_m$ ).

262 The type strain is 29CMI<sup>T</sup> (= CECT 7568<sup>T</sup> =JCM 16414<sup>T</sup> = LMG 25386<sup>T</sup>), isolated  
263 from salted hides. The DNA G+C content of the type strain is 50.7 mol% ( $T_m$ ). This  
264 strain is unable to produce acid from D-galactose, D-mannose or D-mannitol. Able to  
265 utilize D-melibiose, L-raffinose, DL-lysine, L-methionine and L-serine but not maltose,  
266 D-mannose, sucrose, trehalose, xylitol, D-xylose, ethanol, glycerol, propanol, L-alanine,  
267 glutamine, isoleucine, L-ornithine or phenylalanine.

268

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404 Table 1. Characteristics that distinguish strains 29CMI<sup>T</sup> and 53CMI from *Salimicrobium album*  
 405 CCM 3517<sup>T</sup>, *Salimicrobium flavidum* ISL-25<sup>T</sup>, *Salimicrobium halophilum* CCM 4074<sup>T</sup> and  
 406 *Salimicrobium luteum* BY-5<sup>T</sup>. Unless otherwise indicated all data are from this study. +,  
 407 positive; -, negative.

Characteristic	Strain 29CMI <sup>T</sup>	Strain 53CMI	<i>S. album</i> CCM 3517 <sup>T</sup>	<i>S. flavidum</i> ISL-25 <sup>T</sup>	<i>S. halophilum</i> CCM 4074 <sup>T</sup>	<i>S. luteum</i> BY-5 <sup>T</sup>
Cell morphology	Cocci	Cocci	Cocci	Cocci, ovals or rods	Rods	Cocci
Cell size (µm)	1.0-1.5	1.5-2.0	1.0-1.2 <sup>a</sup>	0.4-1.3 × 0.8-9.0 <sup>b</sup>	0.5-1.0 × 2.5-9.0 <sup>c</sup>	0.7-2.4 <sup>d</sup>
Gram	+	+	+	variable	+	+
Endospores	-	-	-	-	+	-
Motility	-	-	+	+	+	-
Colony pigmentation	Yellow	Yellow	White	Pale yellow	Non-pigmented	Yellow
NaCl range (w/v, %)	3-25	3-25	5-20	1-26	3-30	2-27
Optimal NaCl concentration for growth (w/v, %)	7.5-12.5	10	5-15	10	15	10
Nitrate reduction	+	+	+	-	-	-
Urease	-	-	+	-	+	-
Phosphatase	+	+	-	-	-	-
Hydrolysis of:						
Aesculin	-	-	-	-	+	-
DNA	+	+	+	-	+	+
Gelatin	-	-	-	-	-	+
Tween 80	-	-	-	+	+	-
Acid production from:						
D-Fructose	+	+	-	+	-	+
D-Glucose	+	+	-	+	+	+
Maltose	+	+	-	-	-	+

Sucrose	+	+	-	+	+	+
D-Trehalose	+	+	-	+	+	+
D-Xylose	-	-	-	-	+	-
DNA G+C content (mol%)	50.7	51.5	44.9 <sup>a</sup>	49.3 <sup>b</sup>	51.5 <sup>c</sup>	47.9 <sup>d</sup>

408

409 <sup>a</sup> Results from Hao et al. [9].

410 <sup>b</sup> Results from Yoon et al. [33].

411 <sup>c</sup> Results from Ventosa et al. [28].

412 <sup>d</sup> Results from Yoon et al. [32].

413 **Table 2.** Cellular fatty acid composition (%) of strains 29CMI<sup>T</sup>, 53CMI and the species of the  
 414 genus *Salimicrobium*.

415 Strains: 1, *Salimicrobium salexigens* sp. nov. 29CMI<sup>T</sup>; 2, *Salimicrobium salexigens* sp. nov.  
 416 53CMI; 3, *Salimicrobium album* LMG 17430<sup>T</sup> [32]; 4, *Salimicrobium flavidum* ISL-25<sup>T</sup> [33]; 5,  
 417 *Salimicrobium halophilum* KCTC 3566<sup>T</sup> [32]; 6, *Salimicrobium luteum* BY-5<sup>T</sup> [32]. All strains  
 418 were grown under the same conditions (MA medium with 8 % NaCl at 37 °C for 24 h). Fatty  
 419 acids representing <1 % are not shown. -, Not detected.

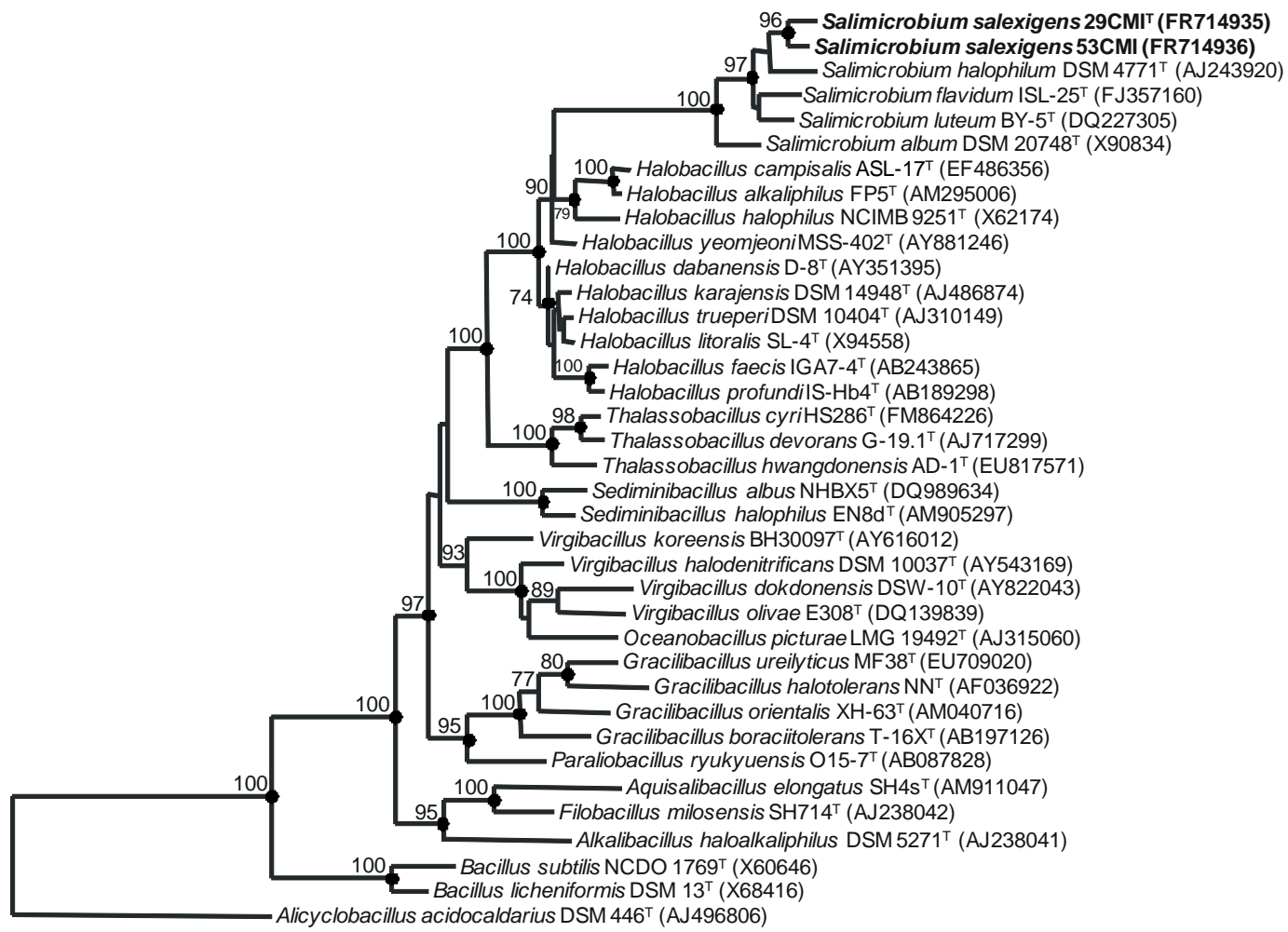
<b>Fatty acid</b>	<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>	<b>6</b>
iso-C <sub>14:0</sub>	3.2	4.7	-	3.4	1.5	2.3
iso-C <sub>15:0</sub>	11.0	6.7	1.8	3.2	37.3	26.1
anteiso-C <sub>15:0</sub>	61.0	63.9	47.0	49.8	24.7	28.7
C <sub>15:0</sub>	-	-	-	1.8	-	1.7
iso-C <sub>16:0</sub>	2.2	2.8	-	10.6	3.4	6.8
C <sub>16:0</sub>	-	1.0	2.4	1.0	1.6	2.4
C <sub>16:1</sub> ω7c	2.2	1.9	-	1.4	1.5	3.0
iso-C <sub>17:0</sub>	1.4	-	2.1	1.4	14.4	11.8
anteiso-C <sub>17:0</sub>	14.6	15.2	29.5	26.4	11.2	14.5
iso-C <sub>17:1</sub> ω10c	-	-	-	-	1.4	-
Summed feature 4*	2.1	1.5	-	-	1.2	1.0
C <sub>18:0</sub>	-	-	4.9	-	-	-
anteiso- C <sub>19:0</sub>	-	-	8.2	-	-	-
C <sub>20:0</sub>	-	-	4.0	-	-	-

420

421 \*Summed feature 4 contains iso-C<sub>17:1</sub> I and/or anteiso-C<sub>17:1</sub> B.

422 **Legend to figures**

423 **Fig. 1.** Maximum-parsimony phylogenetic tree, based on the 16S rRNA gene sequence  
424 comparison, showing the relationship of strains 29CMI<sup>T</sup> and 53CMI with related species  
425 of the genus *Salimicrobium* and other closely related genera of the *Firmicutes*. The  
426 accession numbers of the sequences used in this study are shown in parentheses after  
427 the strain designation. *Alicyclobacillus acidocaldarius* DSM 446<sup>T</sup> was used as outgroup.  
428 Filled circles indicate that the corresponding nodes are also recovered in trees generated  
429 with the neighbour-joining and maximum-likelihood methods. Bootstrap values  $\geq 70\%$   
430 are shown. The scale bar represents 0.01 substitutions per nucleotide position.



0.01

Fig. 1