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[T]) and FR714936 (strain 53CMI).

Abstract

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

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

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

Scope: Systematics

Introduction

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

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

Materials and methods

Isolation and bacterial strains

Strains 29CMI^T and 53CMI were isolated from salted hides obtained from Australia on SW15 medium which contained a mixture of 15% salts [30] after incubation at 37 ºC for 7 days. For routine growth the strains were cultivated in SW10 medium with 10 % (w/v) total salts (8.1 % NaCl, 0.7 % $MgCl_2$, 0.96 % $MgSO_4$, 0.036 % $CaCl_2$, 0.2 % KCl, 0.006 % $NaHCO_3$, 0.0026 % NaBr, 0.5 % yeast extract [Difco]) [30]. The pH was adjusted to 7.5 with 1 M KOH. When necessary, solid media were prepared by adding 2.0 % (w/v) Bacto-agar (Difco). These cultures were maintained at -80 ºC in SW10 medium containing 50 % (v/v) glycerol. The type strains of *Salimicrobium album* CCM 3517^T, *Salimicrobium halophilum* CCM 4074^T, *Salimicrobium luteum* BY-5^T and *Salimicrobium flavidum* ISL-25^T were used for comparative purposes. They were cultivated under the same conditions than strains 29CMI^T and 53CMI.

Phenotypic characterization

The proposed minimal standards for describing new taxa of aerobic, endospore-forming bacteria as recommended by Logan et al. [12] were followed. For the determination of cellular morphology and motility, a culture from liquid 10 % HM medium was examined by light microscopy under a phase-contrast microscope. The morphology of colonies, their size and pigmentation were observed on the 10 % HM solid medium after 48 h of incubation at 37 ºC. The composition of the 10 % HM medium is (w/v): 8.1 % NaCl, 0.7 % $MgCl_2$, 0.96 % $MgSO_4$, 0.036 % $CaCl_2$, 0.2 % KCl, 0.006 % $NaHCO_3$, 0.0026 % NaBr, 0.5 % proteose peptone (Difco), 1.0 % yeast extract (Difco), 0.1 % glucose and 1.5 % agar [30]. Optimal conditions for growth were determined by growing the strains in SW medium at 0, 0.5, 3.0, 5, 7.5, 10, 12.5, 15, 20, 25 and 30 % (w/v) NaCl, and at temperatures of 4, 15, 20, 28, 30, 37, 40 and 45 °C, respectively. The pH range for the isolates was tested in SW10 medium adjusted to the 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 appropriate buffering capacity to each medium [22]. All biochemical tests were carried out at 10 % NaCl and 37 °C, unless it is stated otherwise. Growth under anaerobic conditions was determined by incubating strains in an anaerobic chamber in SW10 medium. Catalase activity was determined by adding a 1 % (w/v) H_2O_2 solution to colonies on SW10 agar medium. Oxidase test was performed using the Dry Slide Assay (Difco). Hydrolysis of aesculin, casein, DNA, gelatin, starch, Tween 80, pullulan and xylan, Voges-Proskauer and methyl red tests, production of indole, arginine, lysine and ornithine decarboxylases, phenylalanine deaminase, phosphatase, urease and nitrate reduction were determined as described by Cowan & Steel [4] with the addition of a 10% total salts to the medium [19,30]. Citrate utilization was determined on Simmon's Citrate medium supplemented with SW10. Acid production from carbohydrates was determined using phenol red base supplemented with 1 % of the carbohydrate and

SW10 medium [30]. For determining the range of substrates used as carbon and energy sources or as carbon, nitrogen and energy sources, the classical medium of Koser [11] as modified by Ventosa et al. [30] was used: 75 g NaCl l^{-1}, 2 g KCl l^{-1}, 0.2 g $MgSO_4 \cdot 7H_2O$ l^{-1}, 1 g KNO_3 l^{-1}, 1 g $(NH_4)_2HPO_4$ l^{-1}, 0.5 g KH_2PO_4 l^{-1} and 0.05 g yeast extract (Difco) l^{-1}. Substrates were added as filter-sterilized solutions to give a final concentration of 1 g l^{-1}, except for carbohydrates, which were used at 2 g l^{-1}. When the substrate was an amino acid, it was tested as carbon, nitrogen and energy source, and the basal medium was therefore prepared without KNO_3 and $(NH_4)_2HPO_4$.

Phylogenetic analysis

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

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

Chemotaxonomic analysis

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

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

Determination of the DNA G+C content and DNA-DNA hybridization

The G+C content of the genomic DNA was determined from the midpoint value of the thermal denaturation profile [15] using the equation of Owen & Hill [18]. DNA-DNA hybridization studies were performed by the competition procedure of the membrane filter method [10]. The hybridization temperature was 51.2 ºC, 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^T 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 ºC 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^T (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^T 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^T and 53CMI was 98.7%. The closest
190 phylogenetic similarity of strains 29CMI^T and 53CMI with other bacterial species was
191 with the type strains of *Salimicrobium album* DSM 20748^T (97.8 % and 97.8 % 16S
192 rRNA sequence similarity, respectively), *Salimicrobium halophilum* DSM 4771^T (97.8
193 % and 97.7 %), *Salimicrobium flavidum* ISL-25^T (97.7 % and 97.9 %) and
194 *Salimicrobium luteum* BY-5^T (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^T, 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^T 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^T and the
204 type strains of the species *Salimicrobium album* CCM 3517^T, *Salimicrobium luteum*
205 BY-5^T, *Salimicrobium flavidum* ISL-25^T and *Salimicrobium halophilum* CCM 4074^T
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^T 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).

The cellular fatty acid profile of strains 29CMI^T and 53CMI was characterized by the fatty acids anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and iso-$C_{14:0}$ as the major fatty acids (Table 2). The fatty acid composition is similar to those found in the other species of *Salimicrobium*, except for the absence of iso-$C_{14:0}$ for *Salimicrobium album* and a higher percentage of iso-$C_{17:0}$ for species *Salimicrobium halophilum* and *Salimicrobium luteum* (Table 2). The cell-wall peptidoglycan analysis showed the presence of *meso*-diaminopimelic acid as diagnostic diamino acid of the peptidoglycan in strains 29CMI^T and 53CMI, indicating that they have the peptidoglycan type A1γ, in accordance with the type reported for other species of the genus *Salimicrobium* [9,28,32,33].

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

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

Description of *Salimicrobium salexigens* sp. nov.

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

Cells are Gram-positive, non-motile, cocci, 1.0-2.0 μm in size, that occur singly, in pairs or tetrads. Colonies are circular, entire, smooth, convex, yellow pigmented and 1-2 mm in diameter on 10 % HM agar medium after 48 h incubation at 37 ºC. Strictly aerobic. Moderately halophilic, growing at 3-25 % (w/v) NaCl; with optimal growth at 7.5-12.5 % (w/v) NaCl. No growth occurs in the absence of NaCl. Grows at 15-40 ºC; showing optimal growth at 37 ºC, and at pH values on the range 5.0-10.0; with optimal growth at pH 7.5. Catalase and oxidase positive. DNA is hydrolysed but gelatin, casein, pullulan, starch, aesculin,Tween 80 and xylan are not hydrolysed. Nitrate is reduced to nitrite. Nitrite not reduced. Acid is produced from D-glucose, D-fructose, maltose, sucrose and D- trehalose but not from D-amygdaline, D-arabinose, arbutine, cellobiose, L-citruline, dulcitol, DL-ethionine, *myo*-inositol, inuline, lactose, melezitose, melibiose, D-ribose, raffinose, sorbitol, xylitol or D-xylose. Indole or H_2S are not produced. Phosphatase is positive. Methyl red, Voges-Proskauer, Simmons' citrate, urease, arginine, lysine and ornithine decarboxylases and phenylalanine deaminase tests are negative. The following compounds are utilized as sole sources of carbon and energy: D-galactose, D-glucose, ribose, D-mannitol, D-sorbitol and acetate. The following compounds are not utilized as sole sources of carbon and energy: D-arabinose, D-cellobiose, D-fructose, D-fucose, lactose, aesculin, D-melizitose, salicin, starch, butanol, dulcitol, *myo*-inositol, methanol, benzoate, citrate, formate, fumarate, hippurate, malate, propionate, succinate, tartrate and valerate. The following compounds are not utilized as sole sources of carbon, nitrogen and energy: L-arginine, aspartate, L-cysteine, L-threonine, tryptophan and valine. The predominat cellular fatty acids are anteiso-$C_{15:0}$, anteiso-$C_{17:0}$, iso-$C_{15:0}$ and iso-$C_{14:0}$. The respiratory isoprenoid quinones are MK-7 (97%) and MK-6 (3%). The polar lipids of the type strain consist of diphosphatidylglycerol, phosphatidylglycerol, one unidentified phospholipid and one

glycolipid. The peptidoglycan type is A1γ, with *meso*-diaminopimelic acid as the diagnostic diamino acid. The DNA G+C content is 50.7-51.5 mol% (T_m).

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

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400 *Salimicrobium luteum* sp. nov. Int. J. Syst. Evol. Microbiol. 57, 2406-2411.

401

402 [33] Yoon, J.-H., Kang, S.-J., Oh, T.-K., Oh, T.-K. (2009) *Salimicrobium flavidum* sp.
403 nov., isolated from a marine solar saltern. Int. J. Syst. Evol. Microbiol. 59, 2839-2842.

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

Characteristic	Strain 29CMI^T	Strain 53CMI	*S. album* CCM 3517^T	*S. flavidum* ISL-25^T	*S. halophilum* CCM 4074^T	*S. luteum* BY-5^T
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[a]	0.4-1.3 × 0.8-9.0[b]	0.5-1.0 × 2.5-9.0[c]	0.7-2.4[d]
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[a]	49.3[b]	51.5[c]	47.9[d]

408

409 [a] Results from Hao et al. [9].

410 [b] Results from Yoon et al. [33].

411 [c] Results from Ventosa et al. [28].

412 [d] Results from Yoon et al. [32].

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

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

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

420

421 *Summed feature 4 contains iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B.

Legend to figures

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

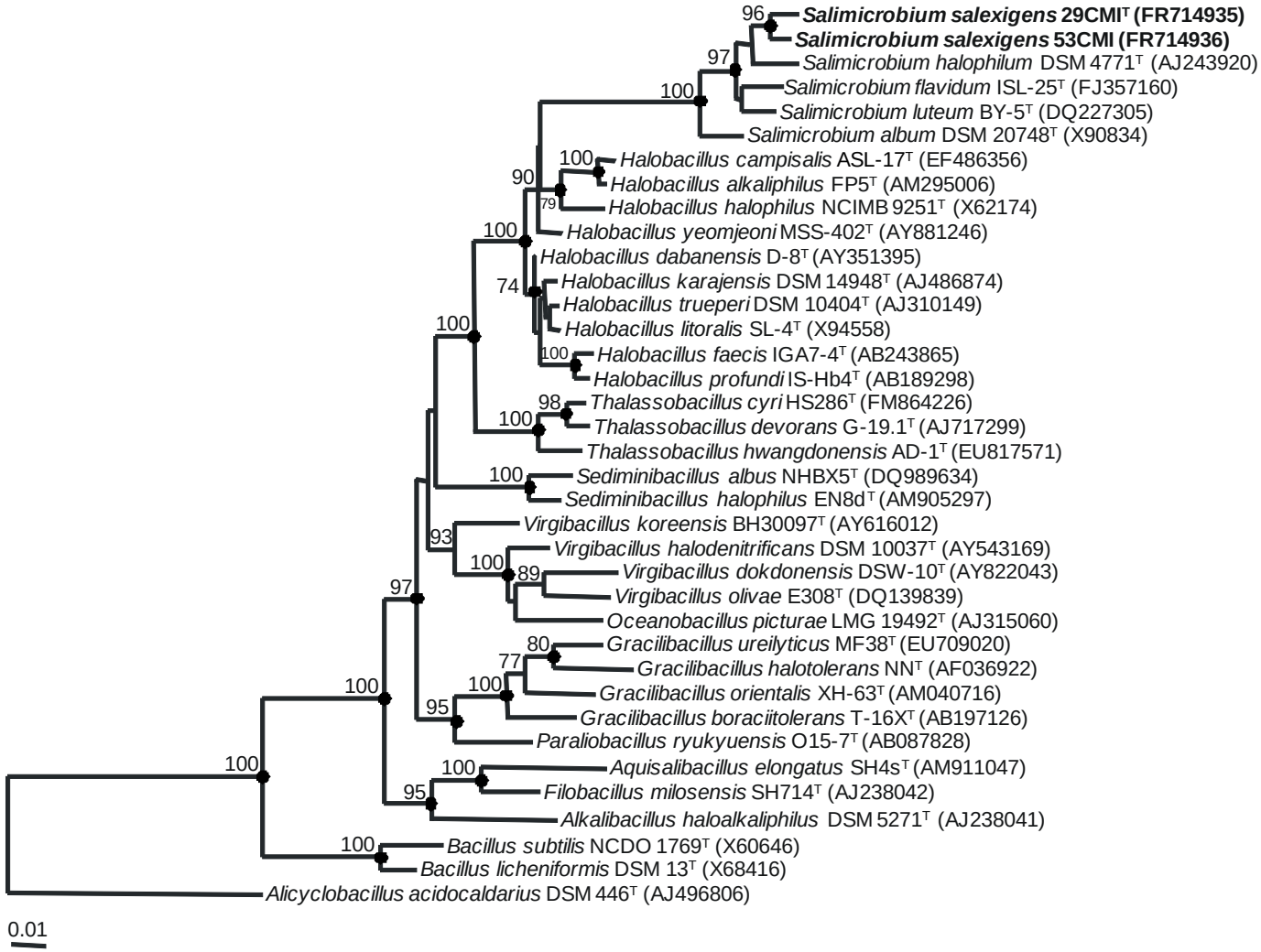
96
Salimicrobium salexigens 29CMI^{T} (FR714935)
Salimicrobium salexigens 53CMI (FR714936)
97
Salimicrobium halophilum DSM 4771^{T} (AJ243920)
100
Salimicrobium flavidum ISL-25^{T} (FJ357160)
Salimicrobium luteum BY-5^{T} (DQ227305)
Salimicrobium album DSM 20748^{T} (X90834)
100
Halobacillus campisalis ASL-17^{T} (EF486356)
90
Halobacillus alkaliphilus FP5^{T} (AM295006)
79
Halobacillus halophilus NCIMB 9251^{T} (X62174)
100
Halobacillus yeomjeoni MSS-402^{T} (AY881246)
Halobacillus dabanensis D-8^{T} (AY351395)
74
Halobacillus karajensis DSM 14948^{T} (AJ486874)
Halobacillus trueperi DSM 10404^{T} (AJ310149)
100
Halobacillus litoralis SL-4^{T} (X94558)
100
Halobacillus faecis IGA7-4^{T} (AB243865)
Halobacillus profundi IS-Hb4^{T} (AB189298)
98
Thalassobacillus cyri HS286^{T} (FM864226)
100
Thalassobacillus devorans G-19.1^{T} (AJ717299)
Thalassobacillus hwangdonensis AD-1^{T} (EU817571)
100
Sediminibacillus albus NHBX5^{T} (DQ989634)
Sediminibacillus halophilus EN8d^{T} (AM905297)
Virgibacillus koreensis BH30097^{T} (AY616012)
93
Virgibacillus halodenitrificans DSM 10037^{T} (AY543169)
100
89
Virgibacillus dokdonensis DSW-10^{T} (AY822043)
97
Virgibacillus olivae E308^{T} (DQ139839)
Oceanobacillus picturae LMG 19492^{T} (AJ315060)
80
Gracilibacillus ureilyticus MF38^{T} (EU709020)
77
Gracilibacillus halotolerans NN^{T} (AF036922)
100
100
Gracilibacillus orientalis XH-63^{T} (AM040716)
95
Gracilibacillus boraciitolerans T-16X^{T} (AB197126)
Paraliobacillus ryukyuensis O15-7^{T} (AB087828)
100
100
Aquisalibacillus elongatus SH4s^{T} (AM911047)
95
Filobacillus milosensis SH714^{T} (AJ238042)
Alkalibacillus haloalkaliphilus DSM 5271^{T} (AJ238041)
100
Bacillus subtilis NCDO 1769^{T} (X60646)
Bacillus licheniformis DSM 13^{T} (X68416)
Alicyclobacillus acidocaldarius DSM 446^{T} (AJ496806)
0.01

Fig. 1