Salinicoccus sesuvii sp. nov., isolated from the rhizosphere of *Sesuvium portulacastrum*

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A Gram-staining-positive coccus, designated CC-SPL15-2^T, was isolated from the rhizosphere of *Sesuvium portulacastrum*. By 16S rRNA gene sequence analysis, it was shown that strain CC-SPL15-2^T belonged to the genus *Salinicoccus*. The isolate was most closely related to *Salinicoccus hispanicus* DSM 5352^T (98.3% 16S rRNA gene sequence similarity) and *Salinicoccus roseus* DSM 5351^T (96.7%); similarities to all other members of the genus *Salinicoccus* were <96.5%. In accordance with characteristics of the genus *Salinicoccus*, the quinone system was mainly composed of menaquinone MK-6. The polar lipid profile exhibited the major components diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. In the polyamine pattern, spermidine was the predominant compound. The fatty acids were anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}, which supported the affiliation of strain CC-SPL15-2^T to the genus *Salinicoccus*. DNA–DNA relatedness between strain CC-SPL15-2^T and *S. hispanicus* CCUG 43288^T was 42 and 32% (reciprocal analysis). From these data as well as from physiological and biochemical tests, a clear differentiation of strain CC-SPL15-2^T from *S. hispanicus* and other members of the genus *Salinicoccus* was possible. We propose that strain CC-SPL15-2^T be assigned to a novel species, with the name *Salinicoccus sesuvii* sp. nov. The type strain is CC-SPL15-2^T (=DSM 23267^T =CCM 7756^T).

Ventosa *et al.* (1990) proposed the genus *Salinicoccus* to contain Gram-staining-positive, catalase- and oxidase-positive, non-motile, non-spore-forming cocci, with MK-6 as the predominant respiratory quinone and a cell wall with peptidoglycan of type L-Lys–Gly₅. At the time of writing, the genus comprises 12 species with validly published names: *S. roseus* and *S. hispanicus* (Ventosa *et al.*, 1990, 1992), *S. alkaliphilus* (Zhang *et al.*, 2002), *S. salsirae* (França *et al.*, 2006), *S. jeotgali* (Aslam *et al.*, 2007), *S. luteus* (Zhang *et al.*, 2007), *S. siamensis* (Pakdeeto *et al.*, 2007), *S. kunmingensis* (Chen *et al.*, 2007), *S. iranensis* (Amoozegar *et al.*, 2008), *S. albus* (Chen *et al.*, 2009), *S. carniancri* (Jung *et al.*, 2010)

and *S. halodurans* (Wang *et al.*, 2008). ‘*S. salitudinis*’ has also been proposed by Chen *et al.* (2008).

Strain CC-SPL15-2^T was isolated from the rhizosphere of *Sesuvium portulacastrum* (sea purslane) in Budai, Chiayi County, Taiwan, on nutrient agar (Oxoid) at 28 °C. The isolate was maintained on tryptone soy agar at 28 °C. Gram staining and cell morphology were observed by phase-contrast microscopy, as described by Kämpfer & Kroppenstedt (2004). Gram staining was also examined by the KOH test (Moaledj, 1986).

The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Multiple sequence alignment and analysis were performed using MEGA version 4 (Tamura *et al.*, 2007). Genetic distances were calculated using distance options according to the Kimura two-parameter model, and clustering using neighbour joining and

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CC-SPL15-2^T is FR669114.

A supplementary figure is available with the online version of this paper.

maximum parsimony was performed using bootstrap values based on 1000 replications. The 16S rRNA gene sequence of strain CC-SPL15-2^T was a continuous stretch of 1487 bp. Sequence similarity indicated that the closest relatives of strain CC-SPL15-2^T were *S. hispanicus* DSM 5352^T (98.3 % 16S rRNA gene sequence similarity) and *S. roseus* DSM 5351^T (96.7 %). Similarities to all other members of the genus *Salinicoccus* were <96.5 %. In the neighbour-joining phylogenetic analysis (Fig. 1), strain CC-SPL15-2^T formed a separate lineage in the genus *Salinicoccus* and clustered with *S. hispanicus* DSM 5352^T.

For analysis of the diagnostic cell-wall diamino acid, polyamines, quinones and polar lipids, cells were grown in PYE medium supplemented with sea salts [0.3 % peptone from casein, 0.3 % yeast extract, 3.1 % sea salts (S9883; Sigma)]. The diagnostic cell-wall diamino acid was extracted and analysed as described by Schleifer (1985). Polyamines were extracted according to Busse & Auling (1988) from biomass harvested at approximately 70 % maximum OD₆₀₀, as reported by Altenburger *et al.* (1997). Polar lipids and quinones were extracted and analysed as described elsewhere (Tindall, 1990a, b; Altenburger *et al.*, 1996). HPLC analysis of quinones and polyamines was carried out using the instrumentation described by Stolz *et al.* (2007).

The diagnostic cell-wall diamino acid was identified to be lysine, which is in accordance with the description of the genus *Salinicoccus* (Ventosa *et al.*, 1990). The polyamine pattern of strain CC-SPL15-2^T consisted of [(g dry weight)⁻¹] 2.2 µmol spermidine, 0.1 µmol spermine and 0.2 µmol putrescine. To the best of our knowledge so far, no

polyamine patterns have been reported for any member of the genus *Salinicoccus*. However, the genus is assigned to the order *Bacillales* and members of other representative genera of this order, such as *Bacillus*, *Paenibacillus*, *Alicyclobacillus* and *Ureibacillus*, always exhibit spermidine as a major compound in the polyamine patterns (Hamana *et al.*, 1989; Hosoya *et al.*, 2004). Hence, high amounts of spermidine in the cells are obviously a common trait of representatives of the order *Bacillales*.

The quinone system of strain CC-SPL15-2^T was composed of menaquinones, with large amounts of MK-6 (99.8 %) and traces of MK-7 (0.2 %). The polar lipid profile of strain CC-SPL15-2^T was composed of major amounts of diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid and minor amounts of two unidentified lipids not stainable with ninhydrin, molybdenum blue or α -naphthol (Supplementary Fig. S1, available in IJSEM Online). The quinone system and polar lipid profile are in excellent agreement with those reported for other members of the genus *Salinicoccus*, but the two unidentified lipids have not been reported previously. In relation to diphosphatidylglycerol and phosphatidylglycerol, the unidentified glycolipid of strain CC-SPL15-2^T showed the same chromatographic behaviour as the unidentified glycolipid detected by Ventosa *et al.* (1992, 1993) in the above-mentioned strains of *S. hispanicus* and *S. roseus*. However, a highly hydrophobic compound is visible in the polar lipid profiles of the type strain of *S. hispanicus* and a strain of *S. roseus* (Ventosa *et al.*, 1992, 1993) which probably corresponds to L1 of strain CC-SPL15-2^T because the chromatographic behaviours are similar.

Fig. 1. Neighbour-joining phylogenetic analysis based on 16S rRNA gene sequences, showing the position of strain CC-SPL15-2^T in the genus *Salinicoccus*. Bootstrap values (>50 %) based on 1000 replications are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid composition of strain CC-SPL15-2^T was similar to those of members of the genus *Salinicoccus* and most similar to the fatty acid composition of *S. hispanicus* CCUG 43288^T, but some quantitative differences were observed (Table 1). The characteristic fatty acids anteiso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0} were detected in strain CC-SPL15-2^T.

The results of the comparative physiological characterization using almost-identical test conditions (Kämpfer *et al.*, 1991) for strain CC-SPL15-2^T and members of the genus *Salinicoccus* are given in Table 2 and the species description. DNA–DNA hybridization between strain CC-SPL15-2^T and *S. hispanicus* CCUG 43288^T was done according to the method of Ziemke *et al.* (1998) and revealed 42 and 32 % (reciprocal analysis) DNA–DNA relatedness.

Our data demonstrate that strain CC-SPL15-2^T exhibits traits that are typical of the genus *Salinicoccus*: for example, the diagnostic diamino acid lysine, a quinone system comprising predominantly menaquinone MK-6 and a polar lipid profile consisting of diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid. The isolate could be distinguished from its closest relative *S. hispanicus* CCUG 43288^T and other members of the genus *Salinicoccus* by physiological traits, DNA–DNA relatedness and differences in 16S rRNA gene sequences. We propose that strain CC-SPL15-2^T is a representative of a novel species, with the name *Salinicoccus sesuvii* sp. nov.

Table 1. Fatty acid compositions of strain CC-SPL15-2^T and its closest phylogenetic neighbour in the genus *Salinicoccus*

Strains: 1, *S. sesuvii* CC-SPL15-2^T; 2, *S. hispanicus* CCUG 43288^T. Data were taken from this study using the TSBA40 database in the MIDI system. ECL, Equivalent chain length. –, Not detected.

Fatty acid (%)	1	2
iso-C _{13:0}	0.7	1.0
iso-C _{14:0}	3.2	2.7
C _{14:0}	15.3	16.5
iso-C _{15:0}	29.6	26.9
anteiso-C _{15:0}	8.6	10.1
C _{15:0}	–	0.7
C _{16:1} ω7c alcohol	0.8	1.0
iso-C _{16:0}	1.3	0.7
Unknown ECL 15.669	10.2	6.1
C _{16:1} ω11c	5.5	12.8
C _{16:0}	13.9	11.8
iso-C _{17:1} ω10c	0.9	1.4
iso-C _{17:0}	2.0	2.4
anteiso-C _{17:0}	0.7	0.9
C _{18:0}	2.5	2.5
iso-C _{19:0}	–	1.0
C _{20:0}	4.3	1.7

Description of *Salinicoccus sesuvii* sp. nov.

Salinicoccus sesuvii [se.su'vi.i. N.L. gen. n. *sesuvii* of *Sesuvium*, isolated from *Sesuvium portulacastrum* L. (sea purslane)].

Gram-staining-positive cocci, about 1.0–1.2 µm in diameter. Oxidase- and catalase-positive; an oxidative metabolism is shown. Good growth occurs after 48 h on tryptone soy agar and marine agar 2218 at 28 °C. No growth occurs on MacConkey agar at 28 °C. No growth occurs at 2 or 45 °C. Growth occurs with 1–25 % (w/v) NaCl [optimum 8–10 % (w/v) NaCl]. Colonies on nutrient agar are smooth, pinkish, circular, translucent and shiny with entire edges. The characteristic diamino acid of the cell wall is lysine. The quinone system is composed mostly of menaquinone MK-6, with traces of MK-7. The polar lipid profile contains major amounts of diphosphatidylglycerol, phosphatidylglycerol and an unidentified glycolipid, and minor amounts of two unidentified lipids. The polyamine pattern contains major amounts of spermidine. The characteristic fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{16:0} and anteiso-C_{17:0}. Physiological tests (including differentiating characters obtained using identical conditions) are indicated in Table 2. Acid is weakly produced from D-glucose and maltose, but no acid is produced from sucrose, adonitol, L-arabinose, D-arabitol, dulcitol, erythritol, *myo*-inositol, lactose, melibiose, methyl α-D-glucoside, raffinose, L-rhamnose, salicin, D-sorbitol or D-xylose. Urease, indole production and hydrolysis of casein, gelatin, starch, DNA and tyrosine are negative. Aesculin hydrolysis and arginine dihydrolase are positive, but H₂S production and lysine decarboxylase, ornithine decarboxylase and β-galactosidase (ONPG) are negative. Only a few compounds are utilized, among them *N*-acetyl-D-glucosamine, D-glucose, maltose, pyruvate and DL-lactate. As sole sources of carbon, D-glucose, maltose, D-mannose, D-fructose and trehalose are utilized, but not acetate, propionate, *N*-acetylgalactosamine, L-arabinose, L-arbutin, cellobiose, D-galactose, gluconate, glycerol, D-mannitol, maltitol, α-melibiose, L-rhamnose, D-ribose, sucrose, salicin, D-xylose, adonitol, *myo*-inositol, D-sorbitol, putrescine, *cis*-aconitate, *trans*-aconitate, 4-aminobutyrate, adipate, azelate, fumarate, glutarate, DL-3-hydroxybutyrate, itaconate, 2-oxoglutarate, suberate, citrate, mesaconate, L-alanine, β-alanine, L-ornithine, L-phenylalanine, L-serine, L-aspartate, L-histidine, L-leucine, L-proline, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate or phenylacetate. The chromogenic substrate *p*-nitrophenyl-β-D-glucopyranoside is hydrolysed weakly, but *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-xylopyranoside, *p*-nitrophenyl-β-D-glucuronide, bis-*p*-nitrophenyl-phosphate, bis-*p*-nitrophenylphenylphosphonate, bis-*p*-nitrophenylphosphorylcholine, 2-deoxythymidine-2'-*p*-nitrophenylphosphate, L-alanine-*p*-nitroanilide, γ-L-glutamate-*p*-nitroanilide and L-proline-*p*-nitroanilide are not hydrolysed.

The type strain is CC-SPL15-2^T (=DSM 23267^T =CCM 7756^T), isolated from the rhizosphere of *Sesuvium portulacastrum*.

Table 2. Differentiating characteristics of strain CC-SPL15-2^T and members of the genus *Salinicoccus*

Strains: 1, *Salinicoccus sesuvii* sp. nov. CC-SPL15-2^T; 2, *S. albus* YIM-Y21^T (Chen *et al.*, 2009); 3, *S. kunmingensis* YIM Y15^T (Chen *et al.*, 2007); 4, *S. alkaliphilus* T8^T (Zhang *et al.*, 2002); 5, *S. roseus* DSM 5351^T (Ventosa *et al.*, 1990); 6, *S. hispanicus* DSM 5352^T (Marquez *et al.*, 1990; Ventosa *et al.*, 1992); 7, *S. jeotgali* S2R53-5^T (Aslam *et al.*, 2007); 8, *S. salsiraiiae* RH1^T (França *et al.*, 2006); 9, *S. siamensis* PN1-2^T (Pakdeeto *et al.*, 2007); 10, *S. luteus* YIM 70202^T (Zhang *et al.*, 2007); 11, *S. iranensis* QW6^T (Amoozegar *et al.*, 2008). All strains possess MK-6 as the predominant respiratory quinone. O, Orange; OP, orange–pink; P, pinkish; PR, pink–red; RO, reddish orange; W, white; Y, yellow; +, positive; tr, trace; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Colony colour	P	W	Y	P	PR	RO	O	PR	O	O	OP
Growth temperature (°C)											
Range	4–36	5–40	4–45	10–49	15–37	15–40	20–30	20–45	15–45	4–45	5–45
Optimum	25–30	25	37	32	37	37	30	37	37	30	35
Growth in NaCl [% (w/v)]											
Range	1–25	1–30	0.5–25	0–25	0.5–25	0.9–25	0.5–15	0–22	1.5–25	1–25	1–25
Optimum	8–10	10	8–10	10	10	10	5	4	10	10	7.5–10
Growth pH											
Range	6.5–11.5	6–10	6–10	6.5–11.5	5–9	6–9	6.5–11	6.5–9.5	6–9	7–11	6.5–10
Optimum	8.5	8.5	8	9.5	7.5	8	7	8	8.5	8–9	7.5
Hydrolysis of:											
Aesculin	+	–	+	+	–	+	+	–	–	+	–
Casein	–	–	–	–	+	–	–	+	–	–	–
Gelatin	+	–	–	–	+	+	–	+	–	–	–
Starch	–	–	+	–	+	–	–	–	–	–	–
Tween 80	–	+	+	–	+	–	–	ND	–	–	–
Nitrate reduced to nitrite	–	+	+	+	+	+	+	+	–	+	+
Indole production	–	–	–	–	–	–	+	–	–	–	+
Methyl red test	–	–	–	–	–	+	+	–	–	–	–
Urease	–	–	–	+	–	+	–	–	–	–	–
Voges–Proskauer test	+	+	–	–	–	–	–	–	–	–	–
Acid production from:											
D-Fructose	–	+	+	–	–	+	+	+	+	–	+
D-Galactose	–	–	–	–	–	+	–	–	–	–	–
D-Glucose	+	+	–	+	–	+	+	+	+	+	+
Glycerol	–	–	–	–	–	+	+	+	+	ND	–
Maltose	+	–	–	–	–	+	+	+	–	+	–
D-Mannitol	–	+	+	+	–	+	–	–	–	ND	+
Sucrose	–	–	+	–	–	+	–	–	–	+	–
Proportion of MK-7 (%)†	0.2	30.8	1.2	–	0.8*	tr*	tr	ND	ND	+	tr

*Data from Chen *et al.* (2007).

†Percentages of total respiratory quinones are given where available. Data for column 6 by Chen *et al.* (2007) were confirmed in this study.

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