Serinicoccus chungangensis sp. nov., isolated from tidal flat sediment, and emended description of the genus *Serinicoccus*

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A Gram-positive, halophilic bacterium, strain CAU 9536^T, was isolated from a tidal flat sediment in the Yellow Sea, Republic of Korea. Strain CAU 9536^T grew optimally at 30 °C, at pH 9.0 and in the presence of 13.0 % (w/v) NaCl. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain CAU 9536^T belonged to the genus *Serinicoccus*, and showed sequence similarity levels of 99.0 % to *Serinicoccus profundus* MCCC 1A05965^T and 98.0 % to *Serinicoccus marinus* JC1078^T. DNA–DNA relatedness values between strain CAU 9536^T and the above two type strains were below 45.0 %. The cell-wall peptidoglycan of strain CAU 9536^T was based on *meso*-diaminopimelic acid, in contrast to those of *S. profundus* and *S. marinus*. The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine and phosphatidylinositol. Whole-cell hydrolysates contained mainly glucose and ribose. The major isoprenoid quinone was menaquinone MK-8 (H₄), and the predominant cellular fatty acid was iso-C_{15:0}. The DNA G + C content of strain CAU 9536^T was 73.5 mol%. Based on phenotypic, chemotaxonomic and genotypic data, strain CAU 9536^T is considered to represent a novel species of the genus *Serinicoccus*, for which the name *Serinicoccus chungangensis* sp. nov. is proposed. The type strain is CAU 9536^T (=KCTC 19774^T=CCUG 59777^T).

The genus *Serinicoccus* was described by Yi *et al.* (2004) and belongs to the family *Intrasporangiaceae*. At the time of writing, the genus comprised two recognized species, *Serinicoccus marinus*, isolated from seawater (Yi *et al.*, 2004), and *Serinicoccus profundus*, isolated from deep-sea sediment (Xiao *et al.*, 2011). Members of the genus are moderately halophilic, Gram-positive cocci, and contain L-ornithine and L-serine as the diagnostic diamino acids in the cell-wall peptidoglycan (Yi *et al.*, 2004). The purpose of this study was to establish the taxonomic position of strain CAU 9536^T, isolated from a tidal flat sediment sample collected from Seogmo Island in the Yellow Sea (12° 55.24' S 76° 09.03' E), Republic of Korea.

Strain CAU 9536^T was isolated by the dilution-plate technique on glucose-yeast extract agar (GYEA; per litre: 10 g yeast extract, 10 g glucose and 15 g agar) (Gordon & Mihm, 1962), supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹) for industrial screening of micro-organisms. Samples were diluted with sterilized distilled water, and serial dilutions were spread onto GYEA

and incubated under aerobic conditions at 30 °C for 3 days. Strain CAU 9536^T was stored at -70 °C in GYE broth supplemented with 25 % (v/v) glycerol. Cell morphology was examined by scanning electron microscopy (JSM-5410LV; JEOL).

Growth at 4, 10, 20, 30, 37, 40 and 45 °C was determined on brain heart infusion agar (BHIA; Difco) in an aerobic incubator (model MIR-253; Sanyo) or anaerobic chamber (model Bactron; Sheldon). The pH range for growth (between pH 5.0 and 11.0 at intervals of 0.5 pH units) and NaCl tolerance (between 0 and 15 % at intervals of 1 %, w/v) were investigated in brain heart infusion broth (BHIB; Difco) at 30 °C for 3 days. A nutrient sporulation medium was used to produce spores (Nicholson & Setlow, 1990). After 3 days growth at 30 °C, endospores were examined by using malachite green staining (Smibert & Krieg, 1994). Catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Oxidase activity was determined based on the oxidation of 1 % (w/v) tetra-methyl-*p*-phenylenediamine (Merck). Enzyme activity was determined with the API ZYM system (bioMérieux), and acid production from glucose and other physiological and biochemical tests were performed with the API 20NE system (bioMérieux), according to the manufacturer's instructions.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CAU 9536^T is HM068886.

Three supplementary figures and a supplementary table are available with the online version of this paper.

Cellular fatty acid methyl esters were extracted after incubation for 3 days in trypticase soy broth (Difco) at 30 °C by acid methanolysis (Minnikin *et al.*, 1980) and were analysed by using an Agilent Technologies 56890N gas chromatograph (Microbial Identification System Instrument) equipped with a cross-linked 5 % phenyl methyl silicon-fused silica capillary column (HP 19091B-102). Menaquinones were analysed as described by Komagata & Suzuki (1987) by using reversed-phase HPLC. Extracted polar lipids were identified by using two-dimensional TLC according to the method of Minnikin *et al.* (1984). Peptidoglycan analysis was performed in our laboratory and by the identification service at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany) as described by Schleifer (1985), with the modification that TLC was used instead of paper chromatography. Whole-cell sugars were analysed by TLC according to the method of Komagata & Suzuki (1987).

Genomic DNA of strain CAU 9536^T was extracted according to the method of Marmur (1961). The 16S rRNA gene was amplified by PCR following established procedures (Cho *et al.*, 2008), and the amplified DNA was sequenced directly by using BigDye Terminator Cycle Sequencing kits (Applied Biosystems) and an automatic DNA sequencer (model 3730; Applied Biosystems). 16S rRNA gene sequences were analysed by using CLUSTAL_X (Thompson *et al.*, 1997) and the EzTaxon server (Chun *et al.*, 2007). Evolutionary distance matrices were generated by using the method described by Jukes & Cantor (1969). A phylogenetic tree was constructed via the neighbour-joining (Saitou & Nei, 1987), least-squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1981) methods from the PHYLIP suite of programs (Felsenstein, 1989). The tree topology was evaluated with the bootstrap resampling method with 1000 replicates (Felsenstein, 1985) of the neighbour-joining dataset with the SEQBOOT and CONSENSE programs from the PHYLIP package. The level of DNA–DNA relatedness between strain CAU 9536^T and the type strains of the two recognized species of the genus *Serinicoccus* was estimated by using the fluorometric microplate method (Ezaki *et al.*, 1989), as modified by Goris *et al.* (1998). The G+C content of the genomic DNA of strain CAU 9536^T was determined by HPLC according to the method of Tamaoka & Komagata (1984), with the modification that the DNA was hydrolysed and the resultant nucleotides were analysed by using reversed-phase HPLC.

Cells of strain CAU 9536^T were Gram-positive, non-sporulating, non-motile, strictly aerobic cocci (see Supplementary Fig. S1 in IJSEM Online). Colonies were yellow with convex and entire margins. Growth was observed on BHIA at 20–40 °C, with optimal growth at 30 °C. The optimum pH for growth was determined in BHIB in a range from pH 5.0 to 11.0, with optimal growth at pH 9.0. Strain CAU 9536^T grew in the presence of 0–15 % (w/v) NaCl, with optimal growth on media containing 13 % (w/v) NaCl.

The phenotypic properties of strain CAU 9536^T are listed in Table 1. The strain was catalase-positive but oxidase-negative; it was positive in tests for nitrate reduction, aesculin hydrolysis, and ferric citrate and capric acid assimilation, but negative in tests for indole production. Acid was not generated from D-glucose. Strain CAU 9536^T gave a negative result in tests for arginine dihydrolase, urease, gelatin hydrolysis and β -galactosidase. It was negative for assimilation of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, adipic acid, malic acid, trisodium citrate and phenylacetic acid. Positive enzyme activities were obtained for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase and β -glucosidase, but not for lipase (C14), β -glucuronidase, α -glucosidase, N-acetyl- β -glucosaminidase, α -mannosidase or α -fucosidase.

The cellular fatty acid profile of strain CAU 9536^T grown on trypticase soy agar comprised C_{15:0} (4.1 % of the total), C_{16:0} (1.2 %), C_{17:0} (2.6 %), iso-C_{14:0} (2.3 %), iso-C_{15:0} (28.3 %), iso-C_{16:0} (16.8 %), iso-C_{17:0} (2.8 %), iso-C_{17:1 ω 9c} (2.7 %), anteiso-C_{15:0} (19.9 %), anteiso-C_{17:0} (8.0 %), C_{17:1 ω 6c} (1.8 %), C_{17:1 ω 8c} (3.4 %) and C_{17:1} 3-OH (1.1 %) (see Supplementary Table S1). Whole-cell

Table 1. Differential phenotypic properties between strain CAU 9536^T and its closest phylogenetic relatives

Strains: 1, CAU 9536^T; 2, *Serinicoccus profundus* DSM 21363^T; 3, *Serinicoccus marinus* KCTC 9980^T; 4, *Ornithinimicrobium humiphilum* DSM 12362^T; 5, *Ornithinimicrobium kibberense* DSM 17687^T; 6, *Kytococcus sedentarius* DSM 20547^T. +, Positive; –, negative; w, weakly positive. All data are from the present study.

Characteristic	1	2	3	4	5	6
Nitrate reduction	+	+	–	+	+	–
Growth with 7 % (w/v) NaCl	+	+	+	–	+	+
Growth at 45 °C	–	–	–	+	–	–
Growth at pH 9.0	+	+	–	–	+	–
Hydrolysis of aesculin	+	+	w	w	w	w
Enzyme activity:						
Acid phosphatase	+	–	–	–	–	w
Alkaline phosphatase	+	+	–	–	–	w
α -Chymotrypsin	+	+	–	w	–	–
Cystine arylamidase	w	+	–	w	w	–
Esterase (C4)	+	+	+	+	w	+
Esterase lipase (C8)	+	+	–	w	–	–
α -Galactosidase	w	–	–	–	–	–
β -Glucosidase	w	+	–	–	–	–
Leucine arylamidase	+	+	–	+	–	w
Naphthol-AS-BI-phosphohydrolase	w	–	w	w	w	w
Trypsin	w	+	–	+	–	–
Valine arylamidase	+	+	+	w	–	–
DNA G+C content (mol%)	73.5	72	72	70	71	69

hydrolysates contained mainly glucose and ribose. The predominant isoprenoid quinone was menaquinone MK8 (H₄). Strain CAU 9536^T had a cell-wall peptidoglycan based on *meso*-diaminopimelic acid as the diagnostic diamino acid (see Supplementary Fig. S2), in contrast to *S. marinus* and *S. profundus*, which have a cell-wall peptidoglycan based on L-ornithine, glutamic acid, alanine, glycine and serine (Yi *et al.*, 2004). The occurrence of *meso*-diaminopimelic acid has only been reported for peptidoglycan type A1 γ and for three variations of peptidoglycan type A4 γ (A31.1, A31.2 and A31.3, see http://www.dsmz.de/microorganisms/main.php?content_id=35). Strain CAU 9536^T had peptidoglycan type A1 γ (A31 according to

http://www.dsmz.de/microorganisms/main.php?content_id=35). The polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidyl-inositol and an unknown lipid (see Supplementary Fig. S3), a slightly different profile from those of *S. marinus* JC1078^T and *S. profundus* MCCC 1A05965^T.

A nearly complete 16S rRNA gene sequence (1511 bp) of strain CAU 9536^T was obtained and compared with the corresponding sequences from other bacterial strains in the GenBank database. Phylogenetic analysis indicated that strain CAU 9536^T belonged to the genus *Serinicoccus* (Fig. 1), and was related most closely to *S. profundus* MCCC

Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences (1511 nt), showing the relationships between strain CAU 9536^T and the type strains of closely related species. Branches of the tree that were also found by using the least-squares and maximum-likelihood algorithms are indicated by asterisks. Branches also obtained by using the maximum-likelihood method are indicated by the letter M. Numbers at nodes indicate levels of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values >50 % are given. Bar, 0.01 substitutions per nucleotide position.

1A05965^T (99.0% 16S rRNA gene sequence similarity), *S. marinus* JC1078^T (98.0%), *Ornithinimicrobium kibberense* K22-20^T (95.0%), *Kytococcus sedentarius* DSM 20547^T (94.7%) and *Ornithinimicrobium humiphilum* DSM 12362^T (94.6%). Levels of DNA–DNA relatedness between strain CAU 9536^T and *S. profundus* MCCC 1A05965^T and *S. marinus* JC1078^T were 40.2 ± 1.2 and 44.1 ± 2.1 %, respectively (mean $\pm$ SD of 5 determinations). These values are below the 70% cut-off recommended by Wayne *et al.* (1987) for the delineation of genomic species, supporting the classification of strain CAU 9536^T as representing a separate species. The DNA G+C content of strain CAU 9536^T was 73.5 mol%.

These data, together with the phenotypic and chemotaxonomic data for strain CAU 9536^T, provide sufficient evidence to support the proposal that this strain represents a novel species of the genus *Serinicoccus*, for which the name *Serinicoccus chungangensis* sp. nov. is proposed.

Description of *Serinicoccus chungangensis* sp. nov.

Serinicoccus chungangensis (chung.ang.en'sis. N.L. masc. adj. *chungangensis* of or belonging to Chung-Ang University, where the taxonomic studies on this species were performed).

Cells are Gram-positive, moderately halophilic, non-sporulating, strictly aerobic, non-motile cocci. Colonies are yellow with convex and entire margins. Growth occurs at 20–40 °C (optimum 30 °C), at pH 5.0–11.0 (optimum pH 9.0) and in the presence of 0–15% (w/v) NaCl (optimum 13%). Positive in tests for catalase, nitrate reduction, aesculin hydrolysis and assimilation of ferric citrate and capric acid. Positive in tests for enzyme activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α -chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -galactosidase and β -glucosidase. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid as the diagnostic diamino acid. The major isoprenoid quinone is menaquinone MK8 (H₄). Whole-cell hydrolysates contain mainly glucose and ribose. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and an unknown lipid. The predominant cellular fatty acids are C_{15:0}, C_{16:0}, C_{17:0}, iso-C_{14:0}, iso-C_{15:0}, iso-C_{16:0}, iso-C_{17:0}, iso-C_{17:1} ω 9c, anteiso-C_{15:0}, anteiso-C_{17:0}, C_{17:1} ω 6c, C_{17:1} ω 8c and C_{17:1} 3-OH.

The type strain, CAU 9536^T (=KCTC 19774^T=CCUG 59777^T), was isolated from a tidal flat sediment sample collected from Seogmo Island in the Yellow Sea, Republic of Korea. The DNA G+C content of the type strain is 73.5 mol%.

Emended description of the genus *Serinicoccus* Yi *et al.* 2004

The description of the genus *Serinicoccus* is as given by Yi *et al.* (2004) and Xiao *et al.* (2011) with the following

amendments. The cell-wall peptidoglycan is based on L-ornithine, glutamic acid, alanine, glycine and serine or *meso*-diaminopimelic acid. The polar lipids are diphosphatidylglycerol, phosphatidylglycerol and phosphatidylinositol. Phosphatidylcholine may be present. The G+C content of the DNA is in the range 72–73.5 mol%.

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