Serinicoccus profundus sp. nov., an actinomycete isolated from deep-sea sediment, and emended description of the genus *Serinicoccus*

Jing Xiao,^{1,2} Yingxue Luo,¹ Shujie Xie¹ and Jun Xu^{1,3}

Correspondence

Jun Xu

xujunn@gmail.com

¹Key Laboratory of Marine Biogenetic Resources, The Third Institute of Oceanography SOA, Xiamen, Fujian 361005, PR China

²Division of Integrative Environmental Sciences, Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

³School of Life Sciences & Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, PR China

A Gram-reaction-positive bacterial strain of the genus *Serinicoccus*, designated MCCC 1A05965^T, was isolated from a deep-sea (5368 m) sediment of the Indian Ocean. Comparison of 16S rRNA gene sequences revealed that the isolate shared 97.6 % sequence similarity with *Serinicoccus marinus* JC1078^T, the type strain of the only described species of the genus *Serinicoccus*. The DNA–DNA relatedness between these two strains was 46.2 % (standard deviation 1.86 %). The cell wall contained alanine, glycine, serine, L-ornithine and glutamic acid, which corresponds to the description of the genus *Serinicoccus*. The acyl type of the glycan chain of the peptidoglycan was glycolyl. Other characteristics of strain MCCC 1A05965^T were consistent with those of the genus *Serinicoccus*. Cells were coccoid, moderately halophilic, oxidase-negative, catalase-positive and non-spore-forming. The major menaquinone was MK-8(H₄). The predominant cellular fatty acids were iso-C_{15:0} (34.7 %) and iso-C_{16:0} (17.0 %). The polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylinositol and an unknown glycolipid. The DNA G + C content was 72 mol%. Strain MCCC 1A05965^T (=0714S6-1^T = DSM 21363^T = CGMCC 4.5582^T) is assigned as the type strain of a novel species, for which the name *Serinicoccus profundus* sp. nov. is proposed.

The genus *Serinicoccus* (family *Intrasporangiaceae*, suborder *Micrococccineae*) was proposed by Yi *et al.* (2004). The genus contains Gram-positive, strictly aerobic, moderately halophilic, oxidase-negative, catalase-positive, non-spore-forming, coccoid bacteria that have L-ornithine and L-serine as the diagnostic amino acids in the peptidoglycan (Yi *et al.*, 2004). *Serinicoccus marinus* is the only described species in the genus. A search of GenBank/EMBL/DDBJ for *Serinicoccus* 16S rRNA gene sequences revealed five sequences: *S. marinus* JC1078^T (Yi *et al.*, 2004) and *Serinicoccus* sp. CNJ927 (Gontang *et al.*, 2007), JL1109 (accession no. DQ985074), JL1131 (DQ985072) and JL1097 (DQ985058), all of which were isolated from marine habitats.

Strain MCCC 1A05965^T was isolated from deep-sea (5368 m) sediment of the Indian Ocean (12° 55.24' S 76° 09.03' E) using the standard dilution-plating tech-

nique on oligotrophic medium (seawater, 2.0 % agar). The isolate was routinely cultured on modified ZoBell 2216E agar (ZBA; 0.5 % tryptone, 0.1 % yeast extract, 3.4 % sodium chloride and 1.8 % agar) at 30 °C and maintained as glycerol suspensions (20 %, v/v) at –80 °C. Biomass for chemical and molecular analyses was prepared from cells cultured in modified ZoBell 2216E broth (ZBB) at 28 °C for 3 days in a rotary shaker and harvested by centrifugation.

The 16S rRNA gene was amplified and sequenced from a single colony using universal primers and conditions as described by Chun & Goodfellow (1995). The almost-complete 16S rRNA gene sequence (1490 nt) of strain MCCC 1A05965^T was compared with sequences in the GenBank and EzTaxon databases using BLAST (Altschul *et al.*, 1997; Chun *et al.*, 2007) and aligned with sequences of representative taxa in GenBank using CLUSTAL X (Thompson *et al.*, 1997). A phylogenetic tree was constructed using the maximum-parsimony and neighbour-joining methods in MEGA version 3.1 (Kumar *et al.*, 2004; Saitou & Nei, 1987).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MCCC 1A05965^T (=0714S6-1^T) is EU603762.

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

The tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) with 1000 replications (Fig. 1). The closest relative of strain MCCC 1A05965^T was *S. marinus* JC1078^T (97.6 % 16S rRNA gene sequence similarity).

Cell motility was examined on ZBA at 30 °C and cell morphology was observed by electron and transmission electron microscopy after cultivation for 3 days. Growth at 5–40 °C (at intervals of 5 °C), at pH 4–12 (at intervals of 1 pH unit) and with 0–15 % (w/v) NaCl (at intervals of 1 %) was determined using ZBA as the basal medium. The Gram reaction was assessed by using the KOH lysis test (Czerny, 1978). Physiological and biochemical tests were performed according to standard procedures (Shirling & Gottlieb, 1966). Hydrolysis of Tween 80 was tested using ZBA as the basal medium. DNase test agar and triple-sugar iron agar (Hangzhou Microbial Reagent Co.) were used to test for DNase and production of H₂S. Biochemical tests, such as nitrate reduction, indole production, acid production from glucose, arginine dihydrolase, urease, β -glucosidase, gelatin hydrolysis, β -galactosidase and some carbohydrate assimilation tests, were performed using the API 20NE system (bioMérieux). Enzyme activities were determined using the API ZYM system (bioMérieux). Carbon source assimilation was tested using the GP2 MicroPlate system (Biolog), according to the manufacturer's instructions. Resistance to various antibiotics was determined with antimicrobial susceptibility discs (Oxoid).

Strain MCCC 1A05965^T was a Gram-reaction-positive, aerobic, moderately halophilic bacterium. Cells were non-motile cocci. The results of biochemical and physiological tests are summarized in the species description.

Triglycerides were extracted and saponified and the resultant fatty acids were esterified as described by Sasser (1990) and determined by using the Microbial Identification System (MIDI) and Sherlock MIS Software. The

cellular fatty acids were C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, iso-C_{16:0}, C_{16:0}, anteiso-C_{17:1} ω 9c, iso-C_{17:1} ω 9c, iso-C_{17:0}, anteiso-C_{17:0}, C_{17:1} ω 8c, C_{17:1} ω 6c and C_{17:0}. Minor differences were found between the fatty acid patterns of strain MCCC 1A05965^T and *S. marinus* JC1078^T (Supplementary Table S1, available in IJSEM Online). Phospholipid analysis was carried out as described by Komagata & Suzuki (1987). The polar lipid pattern consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylcholine and an unidentified glycolipid. Genomic DNA of strain MCCC 1A05965^T was extracted as described by Weisburg *et al.* (1991) and digested and dephosphorylated before determination of the base composition by HPLC analysis (Tamaoka & Komagata, 1984). The molar DNA G+C content was calculated according to Mesbah *et al.* (1989) and found to be 72 mol%. Menaquinones were extracted by the method of Collins *et al.* (1977) and analysed by HPLC (Kroppenstedt, 1985). The major menaquinone of strain MCCC 1A05965^T was MK-8(H₄). DNA–DNA hybridization between strain MCCC 1A05965^T and *S. marinus* DSM 15273^T was performed using the DIG High Prime DNA labelling and detection starter kit II (Roche), according to the manufacturer's directions and Xu *et al.* (2009). DNA–DNA relatedness between strain MCCC 1A05965^T and *S. marinus* DSM 15273^T was 46.2 % (SD 1.86 %).

Analysis of the peptidoglycan structure was performed by the Identification Service of the DSMZ, Braunschweig, Germany, as described by Schleifer (1985) and Schleifer & Kandler (1972), using TLC on cellulose instead of paper. Quantitative analysis of amino acids was carried out by GC and GC/MS according to MacKenzie (1987). The acyl type of the peptidoglycan was determined by using the method of Uchida *et al.* (1999). The total hydrolysate of the peptidoglycan of strain MCCC 1A05965^T contained the amino acids alanine, glycine, serine, L-ornithine and

Fig. 1. Neighbour-joining tree showing the phylogenetic positions of strain 0714S6-1^T (=MCCC 1A05965^T) and representatives of related taxa, based on nearly complete 16S rRNA gene sequences. Bootstrap values based on 1000 replications are shown at branch nodes. The sequence of *Brevibacterium iodinum* NCDO 613^T was used as an outgroup (GenBank accession no. X76567; not shown). Bar, 0.01 substitutions per nucleotide position.

glutamic acid. The identity of the amino acids was confirmed by electron impact MS (320-MS Quadrupole GC/MS; Varian). Quantitative analysis showed that alanine, glycine, serine, ornithine and glutamic acid were present in the ratio 1.5:1.6:0.7:1.0:2.0 in strain MCCC 1A05965^T, which was a slightly different ratio from that observed in this study in *S. marinus* DSM 15273^T (2.2:2.4:0.9:1.0:3.1). Partial hydrolysis (100 °C, 4 M HCl, 45 min) produced the peptide L-Ala-D-Glu and glycine- and/or serine-containing peptides that could not be identified. Dinitrophenylation resulted in dinitrophenylated serine (ether extract) and ornithine (butanol extract), which suggests that serine represents the N terminus of the interpeptide bridge, as in *S. marinus* JC1078^T. Strain MCCC 1A05965^T showed the same peptidoglycan type as *S. marinus* JC1078^T, which is a novel variation of peptidoglycan type A4β. In contrast to *S. marinus* JC1078^T, the acyl type of the peptidoglycan of strain MCCC 1A05965^T was glycolyl.

The morphological traits, DNA G+C content, major menaquinone and occurrence of serine in the peptidoglycan of strain MCCC 1A05965^T corresponded with the characteristics of the genus *Serinicoccus*. However, strain MCCC 1A05965^T can be differentiated from *S. marinus* DSM 15273^T. The polar lipid profile of *S. marinus* DSM 15273^T does not contain phosphatidylcholine. The carbon assimilation (Biolog GP2) and antibiotic susceptibility patterns of the two strains were different (Table 1). Strain MCCC 1A05965^T was resistant to all of the β-lactam antibiotics tested, while *S. marinus* DSM 15273^T was sensitive to those antibiotics. Strain MCCC 1A05965^T produced alkaline phosphatase while *S. marinus* DSM 15273^T did not (API ZYM). The acyl type of the glycan chain of the peptidoglycan was different in strain MCCC 1A05965^T and *S. marinus* DSM 15273^T. Based on the chemotaxonomic and physiological differences and DNA–DNA relatedness, it is proposed that strain MCCC 1A05965^T represents a novel species of the genus *Serinicoccus*, for which we propose the name *Serinicoccus profundus* sp. nov.

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

The genus description is as given by Yi *et al.* (2004) with the addition that phosphatidylcholine may be present.

Description of *Serinicoccus profundus* sp. nov.

Serinicoccus profundus (pro'fun.di. L. gen. n. *profundus* of/ from the depths of the sea).

Gram-reaction-positive, moderately halophilic and aerobic. Oxidase-negative and catalase-positive. No spore formation. Cells are non-motile cocci. Growth occurs with 0–14% (w/v) NaCl (optimum 3–5%), at pH 6–11 (optimum pH 7) and at 10–35 °C (optimum 30 °C). Colonies on ZBA are yellow, circular, convex, entire, glistening, opaque

Table 1. Characteristics that differentiate strain MCCC 1A05965^T from its closest phylogenetic neighbour

Data were obtained in this study.

Characteristic	MCCC 1A05965 ^T	<i>S. marinus</i> DSM 15273 ^T
Phosphatidylcholine	+	–
Alkaline phosphatase	+	–
Utilization of (Biolog GP2):		
Cellobiose	–	+
β-Cyclodextrin	–	+
D-Arabitol	–	+
D-Mannitol	–	+
D-Psicose	–	+
Trehalose	+	–
α-Ketovaleic acid	+	–
Pyruvic acid methyl ester	+	–
Resistance to (μg):		
Ampicillin (10)	+	–
Carbenicillin (100)	+	–
Cefalexin (30)	+	–
Cefazolin (30)	+	–
Cefuroxime (30)	+	–
Cephalothin (30)	+	–
Cephadrin (30)	+	–
Lincomycin (2)	+	–
Oxacillin (1)	+	–
Penicillin G (10)	+	–
Piperacillin (100)	+	–

and butyraceous after 2 days at 28 °C. Does not produce H₂S on triple-sugar iron agar. DNA and Tween 80 are decomposed. With API 20NE, positive for nitrate reduction, aesculin hydrolysis, gelatin decomposition, *p*-nitrophenyl-β-D-galactopyranosidase and utilization of D-glucose, maltose, D-mannose, potassium gluconate and malic acid but negative for indole production, acid production from glucose, arginine dihydrolase, urease and utilization of L-arabinose, D-mannitol, N-acetylglucosamine, adipic acid, capric acid, trisodium citrate and phenylacetic acid. With API ZYM, positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase and α-glucosidase; variable for esterase (C4), trypsin, α-chymotrypsin, cystine arylamidase and β-glucosidase; negative for lipase (C14), acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-fucosidase, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-glucosaminidase and α-mannosidase. With the Biolog GP2 MicroPlate, positive for oxidation of dextrin, glycogen, D-gluconic acid, α-D-glucose, maltose, maltotriose, D-mannose, raffinose, D-ribose, stachyose, sucrose, trehalose, α-hydroxybutyric acid, α-ketovaleic acid, L-lactic acid, pyruvic acid methyl ester, pyruvic acid, L-alaninamide, glycerol, adenosine, inosine, thymidine, uridine, adenosine 5'-monophosphate, thymidine 5'-monophosphate and uridine 5'-monophosphate; weakly positive for oxidation of Tweens 40 and 80, L-arabinose, D-fructose, methyl

α -D-glucoside, palatinose, D-sorbitol, turanose, β -hydroxybutyric acid, γ -hydroxybutyric acid, α -ketoglutaric acid, D-lactic acid methyl ester, L-malic acid, succinic acid monomethyl ester, succinic acid, 2,3-butanediol and 2'-deoxyadenosine. Sensitive to (μ g per disc unless otherwise indicated) bacitracin (0.04 U), chloromycetin (30), ciprofloxacin (5), erythromycin (15), minomycin (30), norfloxacin (10), novobiocin (30), rifampicin (5) and tetracycline (30). Resistant to ampicillin (10), carbenicillin (100), cefalexin (30), cefazolin (30), cefuroxime (30), cephalothin (30), cephradine (30), lincomycin (2), oxacillin (1), penicillin G (10) and piperacillin (100). The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylcholine and an unknown glycolipid. The major fatty acids are iso-C_{15:0}, iso-C_{16:0}, anteiso-C_{15:0}, iso-C_{17:0} and anteiso-C_{17:0}. The major menaquinone is MK-8(H₄). The DNA G+C content of the type strain is 72 mol%.

The type strain, MCCC 1A05965^T (=0714S6-1^T =DSM 21363^T =CGMCC 4.5582^T), was isolated from a deep-sea sediment.

Acknowledgements

J. Xu is indebted to the National Science Foundation of China (grant no. 40606031), the National High Technology R&D Program of China (grant no. 2007AA091904) and the China Ocean Mineral Resources Research and Development Association (grant no. DYXM-115-02-2-8).

References

- Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.
- Chun, J. & Goodfellow, M. (1995). A phylogenetic analysis of the genus *Nocardia* with 16S rRNA gene sequences. *Int J Syst Bacteriol* **45**, 240–245.
- Chun, J., Lee, J. H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.
- Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.
- Czerny, G. (1978). Studies on aminopeptidase for the distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 113–122.
- Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.
- Gontang, E. A., Fenical, W. & Jensen, P. R. (2007). Phylogenetic diversity of gram-positive bacteria cultured from marine sediments. *Appl Environ Microbiol* **73**, 3272–3282.
- Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Kroppenstedt, R. M. (1985). Fatty acid and menaquinone analysis of actinomycetes and related organisms. In *Chemical Methods in Bacterial Systematics* (Society for Applied Bacteriology Technical Series vol. 20), pp. 173–199. Edited by M. Goodfellow & D. E. Minnikin. New York: Academic Press.
- Kumar, S., Tamura, K. & Nei, M. (2004). MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* **5**, 150–163.
- MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyryl isobutyl esters. *J Assoc Off Anal Chem* **70**, 151–160.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *USFCC Newsl* **20**, 16.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Shirling, E. B. & Gottlieb, D. (1966). Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* **16**, 313–340.
- Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Uchida, K., Kudo, T., Suzuki, K. & Nakase, T. (1999). A new rapid method of glycolate test by diethyl ether extraction, which is applicable to a small amount of bacterial cells of less than one milligram. *J Gen Appl Microbiol* **45**, 49–56.
- Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.
- Xu, J., Wang, Y., Xie, S. J., Xu, J., Xiao, J. & Ruan, J. S. (2009). *Streptomyces xiamenensis* sp. nov., isolated from mangrove sediment. *Int J Syst Evol Microbiol* **59**, 472–476.
- Yi, H., Schumann, P., Sohn, K. & Chun, J. (2004). *Serinicoccus marinus* gen. nov., sp. nov., a novel actinomycete with L-ornithine and L-serine in the peptidoglycan. *Int J Syst Evol Microbiol* **54**, 1585–1589.