Serinicoccus marinus gen. nov., sp. nov., a novel actinomycete with L-ornithine and L-serine in the peptidoglycan

Hana Yi,¹ Peter Schumann,² Kyounghee Sohn¹ and Jongsik Chun¹

Correspondence
Jongsik Chun
jchun@snu.ac.kr

¹School of Biological Sciences, Seoul National University, 56-1 Shillim-dong, Kwanak-gu, Seoul 151-742, Republic of Korea

²DSMZ – German Collection of Microorganisms and Cell Cultures, Mascheroder Weg 1b, D-38124 Braunschweig, Germany

A Gram-positive bacterial strain containing L-ornithine as the diagnostic diamino acid was isolated from a sea-water-sample from the East Sea, Korea. A phylogenetic analysis based on 16S rRNA gene sequences showed that strain JC1078^T represents a phyletic line within the suborder *Micrococcineae* of the order *Actinomycetales*, adjacent to the genus *Ornithinimicrobium*. The highest sequence similarity values to the isolate were observed against *Ornithinimicrobium humiphilum* (94.3 %) and *Kytococcus sedentarius* (94.1 %). The strain was strictly aerobic and moderately halophilic with optimal growth at 2–3 % (w/v) NaCl. Cells were non-motile, non-sporulating and coccoid-shaped. The cell wall contains L-ornithine, glutamic acid, alanine, glycine and serine. The major menaquinone was MK-8(H₄). The predominant cellular fatty acids were of the iso- and anteiso-methyl-branched types. The polar lipids were phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and an unknown glycolipid. The acyl type of the glycan chain of peptidoglycan is acetyl. The DNA G + C content was 72 mol%. The combination of physiological, biochemical and chemotaxonomical data clearly separated the marine isolate from other members of the suborder *Micrococcineae*. On the basis of polyphasic evidence, it is proposed to classify strain JC1078^T in a novel genus and species, for which the name *Serinicoccus marinus* gen. nov., sp. nov. is proposed. The type strain is JC1078^T (=IMSNU 14026^T = KCTC 9980^T = DSM 15273^T).

Actinomycetes containing ornithine as the diagnostic diamino acid in their peptidoglycans are phylogenetically assigned to the families *Cellulomonadaceae* (*Cellulomonas*, *Rarobacter*) and *Microbacteriaceae* (*Microbacterium*, *Curto-bacterium*) within the suborder *Micrococcineae* (Stackebrandt *et al.*, 1997). In addition, two genera, namely *Ornithinimicrobium* and *Ornithinimicrobium*, have been proposed to harbour organisms containing ornithine in the peptidoglycan (Groth *et al.*, 1999, 2001). The differentiation of these two genera was based on the peptidoglycan structure, polar lipid pattern, fatty acid profile and morphology as well as 16S rRNA gene analysis (Groth *et al.*, 2001). In this study, we report the polyphasic characterization of a marine isolate containing ornithine in the peptidoglycan and propose to classify the organism in a novel genus and species, for which the name *Serinicoccus marinus* gen. nov., sp. nov. is proposed.

Strain JC0178^T was isolated from a surface sea-water-sample collected from the East Sea using the standard dilution plating technique. Isolation was achieved using Marine agar 2216 (MA; Difco) and incubation at 30 °C. The isolate was routinely cultured on MA and maintained as a glycerol suspension (20 %, w/v) at –80 °C.

The 16S rRNA gene was enzymically amplified from a single colony. Primers, PCR conditions and sequencing were described elsewhere (Chun & Goodfellow, 1995). The sequence of strain JC1078^T was manually aligned with representative sequences of actinomycetes obtained from the GenBank database. Phylogenetic trees were inferred by using the Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981), maximum-parsimony (Fitch, 1972) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices for the neighbour-joining and Fitch–Margoliash methods were generated according to the model of Jukes & Cantor (1969). The resultant neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. The alignment and phylogenetic analyses were carried out using the PHYLIT program

Published online ahead of print on 5 March 2004 as DOI 10.1099/ij.s.0.03036-0.

The GenBank accession number for 16S rRNA gene sequence of strain JC1078^T is AY382898.

(available at <http://plaza.snu.ac.kr/~jchun/phydit/>) and PAUP 4.0 (Swofford, 1998) as described (Chun *et al.*, 2000).

A nearly complete 16S rRNA gene sequence of strain JC1078^T was obtained (1420 bp). Preliminary sequence comparison against the 16S rRNA gene sequences deposited in the GenBank database indicated that our isolate belonged to the suborder *Micrococcineae* in the order *Actinomycetales*. On the basis of 16S rRNA gene similarity, the closest cultured relatives were *Ornithinimicrobium humiphilum* DSM 12362^T (94.3%), *Kytococcus sedentarius* DSM 20547^T (94.1%), *Demetria nishinomiyaensis* DSM 20448^T (93.9%), *Arthrobacter cummingsii* DSM 10493^T (93.7%), *Demetria terragena* DSM 11295^T (93.7%), *Arthrobacter albus* CF-43^T (93.5%) and *Janibacter limosus* DSM 11140^T (93.1%). This relationship between our isolate and other members of the suborder *Micrococcineae* was also evident in the phylogenetic trees (Fig. 1). Strain JC1078^T and *Ornithinimicrobium humiphilum* DSM 12362^T formed a monophyletic clade with a relatively low bootstrap value (49%) which was supported by all tree-making methods employed in this study. The positions of the other taxa that showed more than 93% 16S rRNA gene sequence similarities to our marine isolate varied depending on the inferring algorithm.

Cellular morphology and motility were examined after growth on MA at 30 °C for 3 days by scanning electron and phase-contrast microscopy, respectively. The strain was checked for the formation of mycelium and spores by growing on several culture media, namely MA, ISP medium #3 (Difco), ISP medium #4 (Difco), PYGV (Staley, 1968) supplemented by artificial sea water (ASW; Lyman & Fleming, 1940), nutrient agar (Difco) supplemented by ASW and basal medium (BM; Baumann *et al.*, 1972).

Growth under anaerobic conditions was determined in an anaerobic chamber (10% CO₂, 10% H₂, 80% N₂; Sheldon Manufacturing). The growth range for temperature (between 5 and 50 °C with intervals of 5 °C), pH (between

pH 4 and 12 with intervals of 1) and NaCl concentration [between 0 and 15% (w/v) with intervals of 1%] was determined using synthetic ZoBell medium (Zobell, 1941); growth was checked after up to 4 weeks.

Standard physiological and biochemical tests were performed as described previously (Smibert & Krieg, 1994). Acid-fastness was determined by Ziehl-Neelsen staining. Hydrolysis of alginic acid, casein, cellulose, chitin, starch and Tween 80 was tested using MA as the basal medium. DNase test agar (Difco) supplemented with 2.5% (w/v) NaCl was used for DNase assay. Decomposition of adenine, hypoxanthine, L-tyrosine and xanthine was tested using MA according to Gordon *et al.* (1974). Production of H₂S was detected in triple-sugar iron agar (TSI) supplemented with 2.5% (w/v) NaCl. Arginine dihydrolase, β -galactosidase, nitrate reduction, urease, acid production from glucose and indole production tests were performed using an API 20NE kit (bioMérieux), and other enzymic activities were determined using an API ZYM kit (bioMérieux). Strips were inoculated with a heavy bacterial suspension in half-strength ASW and AUX medium supplemented with 2.5% (w/v) NaCl. β -Galactosidase activity was additionally determined by streaking cultures onto MA agar plates amended with 0.1 mM IPTG and 20 μ g X-Gal ml⁻¹ (Gosink *et al.*, 1998). Carbon source utilization was tested in 96-well tissue culture microplates as described by Gosink *et al.* (1998) using BM supplemented with 1% (v/v) of vitamin solution (Staley, 1968) as basal medium.

The results of biochemical and physiological tests are presented in the genus and species description.

Extraction of fatty acid methyl esters and their gas chromatographic separation were performed by using the Microbial Identification System according to the instructions in the operating manual (MIDI, 1999). The peptidoglycan structure was elucidated by analysis of purified cell-wall hydrolysates according to Schleifer (1985). The amino acids and peptides were analysed by two-dimensional

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain JC1078^T and members of the suborder *Micrococcineae*. The percentage numbers at the nodes are the levels of bootstrap support based on neighbour-joining analyses of 1000 resampled datasets, and solid circles indicate that the corresponding nodes (groupings) are also recovered in Fitch-Margoliash, maximum-likelihood and maximum-parsimony trees. *Helicobacter pylori* ATCC 43504^T (M88157) was used as an out-group. Scale bar, 0.1 nucleotide substitutions per position.

ascending TLC on cellulose plates using the solvent systems of Schleifer & Kandler (1972). The molar ratios of amino acids were determined by GC and GC/MS of *N*-heptafluorobutyl amino acid isobutyl esters (MacKenzie, 1987; Groth *et al.*, 1996). The amino terminal amino acid of the interpeptide bridge was determined by dinitrophenylation as described by Schleifer (1985). The glycolate content of bacterial cell walls was determined by the colorimetric method of Uchida & Aida (1984). Menaquinones were extracted according to Collins *et al.* (1977) and were analysed by HPLC (Groth *et al.*, 1996).

DNA for determination of the G+C value was isolated using a French pressure cell (ThermoSpectronic) and purified on hydroxyapatite as described by Cashion *et al.* (1977). The G+C content was determined by reverse-phase HPLC of nucleosides according to Mesbah *et al.* (1989).

The peptidoglycan of strain JC1078^T contained ornithine, alanine, glycine, serine and glutamic acid in an approximate molar ratio of 1:2:2:1:3. Labelling by 1-fluoro-2,4-dinitrobenzene revealed that serine represents the amino terminus of the interpeptide bridge. Dinitrophenylated glutamic acid could not be detected. The peptide L-Ala-D-Glu typical of peptidoglycans of the A-type of cross-linkage was found in the partial hydrolysate (4M HCl, 100 °C, 45 min). Because the results of the peptidoglycan analysis are inconsistent with all hitherto published structures of peptidoglycans containing ornithine and serine (<http://www.dsmz.de/species/murein.htm>) and because of the phylogenetic proximity to *Ornithinococcus hortensis* and *Ornithinimicrobium humiphilum*, it is likely that strain JC1078^T represents a novel variation of peptidoglycan type A4β. However, a detailed peptidoglycan structure can not be concluded from the available data. The acyl type of the glycan chain of peptidoglycan is acetyl. The major menaquinone of strain JC1078^T was MK-8(H₄). The polar lipid pattern consisted of phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and an unidentified glycolipid. The cellular fatty acids were i-14:0 (1.30%), i-15:1 (1.88%), i-15:0 (41.65%), ai-15:0 (8.18%), 15:0 (0.89%), i-16:0 (0.79%), i-16:0 (12.83%), 16:0 (1.79%), i-17:1ω9c (12.21%), i-17:0 (9.24%), ai-17:0 (6.96%), 17:1ω8c (1.00%) and 17:0 (1.28%). The DNA base composition of strain JC1078^T was 72 mol% G+C.

Strain JC1078^T corresponds to its phylogenetic neighbour *Ornithinimicrobium humiphilum* and to *Ornithinococcus hortensis* in displaying an A-type peptidoglycan based on ornithine, in the DNA base composition of approximately 70 mol% G+C and in the major menaquinone MK-8(H₄). However, strain JC1078^T can be differentiated from both genera by the occurrence of serine in the peptidoglycan and by its polar lipid pattern, fatty acid profile and physiological characteristics as shown in Table 1. All other phylogenetically related genera of the suborder *Micrococcineae* can be differentiated easily from strain JC1078^T by phenotypic characteristics such as the peptidoglycan structure. Based on the differentiation from all validly described

genera of the suborder *Micrococcineae* by phylogenetic analysis, chemotaxonomic investigations and physiological studies, it is proposed to classify the new isolate in a novel genus and species, *Serinicoccus marinus* gen. nov., sp. nov., with the type strain JC1078^T.

Description of *Serinicoccus* gen. nov.

Serinicoccus (Ser.in'i.coc.cus. N.L. n. *serinum* serine; N.L. masc. n. *coccus* from Gr. masc. n. *kokkos* a grain, seed; N.L. masc. n. *Serinicoccus* a coccus with serine in the cell wall).

Gram-positive, strictly aerobic, moderately halophilic bacteria. Oxidase-negative, catalase-positive, not acid-fast. No formation of spores. Cells are non-motile cocci. The peptidoglycan type is of the A type of cross-linkage and contains ornithine, alanine, glycine, serine and glutamic acid in an approximate molar ratio of 1:2:2:1:3. The acyl type of the glycan chain of peptidoglycan is acetyl. The major menaquinone is MK-8(H₄). The major cellular fatty acids are of the iso- and anteiso-methyl-branched type. The polar lipids are phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol and one unknown glycolipid. The DNA G+C content is 72%. Phylogenetically, this genus is affiliated to the suborder *Micrococcineae*. The type species is *Serinicoccus marinus*.

Description of *Serinicoccus marinus* sp. nov.

Serinicoccus marinus (ma.ri'nus. L. masc. adj. *marinus* of or belonging to the sea, marine).

In addition to the characteristics that define the genus, it has the characteristics described below. Growth occurs at NaCl concentrations of 0–14% (w/v), the optimum being 2–3%. The pH range for growth is between pH 6 and 11, the optimum being pH 8. The temperature range for growth is 10–35 °C, with the optimum being 35 °C. Extended incubation (up to 20 days) is required at 5 °C. Cells are cocci with diameters of 0.5–0.9 μm. Colonies on MA are yellow, circular, convex, entire, glistening, opaque and butyraceous. Colonies are approximately 1 mm in diameter after 3 days (on MA at 30 °C) and reach the maximum diameter of 3–4 mm after 5 days. Nitrate is reduced to nitrite. Does not show arginine dihydrolase activity. β-Galactosidase activity is weakly present. Does not produce H₂S on TSI agar, acid from glucose in API 20NE kit or indole from tryptophan. Casein, DNA, aesculin, gelatin, starch, Tween 80 and tyrosine are decomposed; adenine, alginate, cellulose, chitin, hypoxanthine, urea and xanthine are not. Tested by the API ZYM enzyme assay, strain JC1078^T is positive for esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and α-glucosidase; variable for trypsin and β-glucosidase; negative for alkaline phosphatase, lipase (C14), cystine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase or α-fucosidase. Utilizes acetate, D-cellobiose, D-fructose,

Table 1. Major characteristics that differentiate strain JC1078^T from phylogenetically related ornithine-containing bacteria

Strains: 1, JC1078^T; 2, *Ornithinimicrobium humiphilum* DSM 12362^T; 3, *Ornithinococcus hortensis* DSM 12335^T. Data are from this and earlier studies (Groth *et al.*, 1999, 2001). Abbreviations: Orn, ornithine; Ala, alanine; Gly, glycine; Glu, glutamate; Ser, serine; Asp, aspartate; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; PI, phosphatidylinositol; PSer, phosphatidylserine; PLs, unknown phospholipids; GL(s), unknown glycolipid(s); I, iso-methyl-branched; A, anteiso-methyl-branched; S, straight chain saturated. Fatty acid type: fatty acids which show more than 5 % of total composition are considered.

Characteristic	1	2	3
Cell morphology	Cocci	Cocci, irregular rods	Cocci
Growth in the presence of 12 % NaCl	+	–	–
Growth at 45 °C	–	+	–
Wall amino acids	Orn, Ala, Gly, Glu, Ser	Orn, Ala, Gly, Glu, Asp	Orn, Ala, Gly, Glu
Polar lipid type	PG, DPG, PI, GL	PG, DPG, PI, PLs, GLs	PG, DPG, PI, PLs, PSer
Fatty acid type	I, A	I, A	I, A, S
G + C content (mol%)	72	70	72
Nitrate reduction	–	+	+
H ₂ S production	–	+	+
Decomposition of:			
Aesculin	+	–	–
Hypoxanthine	–	+	+
Starch	+	+	–
Tween 80	+	–	+
Tyrosine	+	–	–
Xanthine	–	+	+
API ZYM:			
Alkaline phosphatase	–	+	+
Lipase (C14)	–	–	w
Cystine arylamidase	–	+	+
α-Chymotrypsin	–	w	+
Acid phosphatase	–	+	w
Naphthol-AS-BI-phosphohydrolase	–	+	+

D-galactose, D-glucose, D-mannitol, D-mannose, D-sorbitol, D-trehalose, glycerol and sucrose as a sole carbon source. Does not utilize acetamide, benzoate, citrate, D-ribose, ethanol, glycine, inulin, 2-propanol, lactose, L-arginine, L-ascorbate, L-lysine, L-rhamnose, *N*-acetylglucosamine, polyethylene glycol, salicylate or tartrate. The major fatty acids are i-15:0, i-16:0, i-17:1ω9c, i-17:0, ai-15:0 and ai-17:0. The DNA G + C content is 72 mol%.

The type strain, JC1078^T (=IMSNU 14026^T=KCTC 9980^T=DSM 15273^T), was isolated from a sea-water sample from the East Sea, Korea.

Acknowledgements

We are grateful to Dr J. P. Euzéby for the help with nomenclature. This work was supported by the 21C Frontier Microbial Genomics and Applications Center Program (grant MG02-0101-001-2-1-0) and the Strategic National R&D Program through the Genetic Resources and Information Network (grant M1-0219-00-0018), MOST, Republic of Korea.

References

Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

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., Bae, K. S., Moon, E. Y., Jung, S. O., Lee, H. K. & Kim, S. J. (2000). *Nocardiopsis kunsanensis* sp. nov., a moderately halophilic actinomycete isolated from a saltern. *Int J Syst Evol Microbiol* **50**, 1909–1913.

Collins, M. D., Pirouz, T., Goodfellow, M. & Minnikin, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* **100**, 221–230.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fitch, W. M. (1972). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Fitch, W. M. & Margoliash, E. (1967). Construction of phylogenetic trees. *Science* **155**, 279–284.

Gordon, R. E., Barnett, D. A., Handerman, J. E. & Pang, C. H.-N. (1974). *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Sys Bacteriol* **24**, 54–63.

Gosink, J. J., Woese, C. R. & Staley, J. T. (1998). *Polaribacter* gen. nov., with three new species, *P. irgensii* sp. nov., *P. franzmannii* sp.

nov. and *P. filamentus* sp. nov., gas vacuolate polar marine bacteria of the *Cytophaga-Flavobacterium-Bacteroides* group and reclassification of '*Flectobacillus glomeratus*' as *Polaribacter glomeratus* comb. nov. *Int J Syst Bacteriol* **48**, 223–235.

Groth, I., Schumann, P., Weiss, N., Martin, K. & Rainey, F. A. (1996). *Agrococcus jenensis* gen. nov., sp. nov., a new genus of actinomycetes with diaminobutyric acid in the cell wall. *Int J Syst Bacteriol* **46**, 234–239.

Groth, I., Schumann, P., Martin, K., Schuetze, B., Augsten, K., Kramer, I. & Stackebrandt, E. (1999). *Ornithinicoccus hortensis* gen. nov., sp. nov., a soil actinomycete which contains L-ornithine. *Int J Syst Bacteriol* **49**, 1717–1724.

Groth, I., Schumann, P., Weiss, N., Schuetze, B., Augsten, K. & Stackebrandt, E. (2001). *Ornithinimicrobium humiphilum* gen. nov., sp. nov., a novel soil actinomycete with L-ornithine in the peptidoglycan. *Int J Syst Evol Microbiol* **51**, 81–87.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Lyman, J. & Fleming, R. H. (1940). Composition of sea water. *J Mar Res* **3**, 134–146.

MacKenzie, S. L. (1987). Gas chromatographic analysis of amino acids as the *N*-heptafluorobutyl 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.

MIDI (1999). *Sherlock, Microbial Identification System, Operating Manual*, version 3.0. Newark, DE: MIDI, Inc.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

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.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Washington, DC: American Society for Microbiology.

Stackebrandt, E., Rainey, F. A. & Ward-Rainey, N. L. (1997). Proposal for a new hierarchic classification system, *Actinobacteria* classis nov. *Int J Syst Bacteriol* **47**, 479–491.

Staley, J. T. (1968). *Prosthecomicrobium* and *Ancalomicrobium*: new prosthecate freshwater bacteria. *J Bacteriol* **95**, 1921–1942.

Swofford, D. L. (1998). *Phylogenetic Analysis Using Parsimony (PAUP)*. Version 4. Sunderland, MA: Sinauer Associates.

Uchida, K. & Aida, K. (1984). An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* **30**, 131–134.

Zobell, C. E. (1941). Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. *J Mar Res* **4**, 42–75.