Serinibacter salmoneus gen. nov., sp. nov., an actinobacterium isolated from the intestinal tract of a fish, and emended descriptions of the families *Beutenbergiaceae* and *Bogoriellaceae*

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A novel Gram-positive bacterium, designated Kis4-28^T, was isolated from the intestinal tract of a fish, and its taxonomic position was investigated by a polyphasic approach. The sample was collected from the coast of Tokyo Bay, Japan. Cells of strain Kis4-28^T were rod-shaped, non-motile and non-sporulating. The peptidoglycan type of the isolate was A4 α ; lysine was the diagnostic diamino acid. The only menaquinone detected was MK-8(H₄), and the major fatty acids were anteiso-C₁₅:0 and C₁₆:0. Galactose was detected as a major cell-wall sugar. The polar lipids were phosphatidylethanolamine and phosphatidylglycerol. The DNA G+C content was 70.7 mol%. 16S rRNA gene sequence analysis revealed that strain Kis4-28^T and the type strain of *Salana multivorans* formed a monophyletic cluster with a 16S rRNA gene sequence similarity of 96.2%. Strain Kis4-28^T was clearly distinguishable from the genus *Salana* in terms of its chemotaxonomic characteristics. On the basis of the genotypic and phenotypic characteristics, a new genus and species is proposed for strain Kis4-28^T, with the name *Serinibacter salmoneus* gen. nov., sp. nov. The type strain of *Serinibacter salmoneus* is Kis4-28^T (=NBRC 104924^T =DSM 21801^T). In addition, on the basis of 16S rRNA gene sequence analysis of the genus *Serinibacter* and related genera, emended descriptions of the families *Beutenbergiaceae* and *Bogoriellaceae* are proposed to accommodate the genera *Beutenbergia*, *Salana* and *Serinibacter*, and the genera *Bogoriella* and *Georgenia*, respectively.

The suborder *Micrococcineae* was proposed by Stackebrandt *et al.* (1997). So far, 15 families have been assigned to this suborder (Stackebrandt *et al.*, 1997; Stackebrandt & Schumann, 2000; Li *et al.*, 2005, 2008; Zhi *et al.*, 2009). Of them, the genera *Beutenbergia* (Groth *et al.*, 1999), *Salana* (von Wintzingerode *et al.*, 2001) and *Georgenia* (Altenburger *et al.*, 2002) have been proposed as the members of the family *Beutenbergiaceae* (Zhi *et al.*, 2009). It has been reported that the genus *Ruania* is phylogenetically related to the genera *Georgenia*, *Bogoriella* (Groth *et al.*, 1997), *Beutenbergia* and *Salana*, but the family to which the genus *Ruania* belongs has not been specified (Gu *et al.*, 2007).

Members of the suborder *Micrococcineae* are widespread and occur in both natural and artificial habitats, e.g. in soil, plants, sediments, food and dairy products. While isolating bacteria from marine samples, we isolated a novel actinobacterium (strain Kis4-28^T) from the intestinal tract of a fish species collected from Tokyo Bay, Japan. Comparative 16S rRNA gene sequence analysis revealed that strain Kis4-28^T is a member of the suborder *Micrococcineae*. The objective of this study was to determine the taxonomic position of this strain by using a polyphasic approach.

Japanese sillago (*Sillago japonica*), a type of fish, was collected from Kyonan beach on the coast of Tokyo Bay, Japan. The sillago was dissected, and an intestinal tract sample of approximately 1 g was used for the isolation of bacteria. The procedure employed for bacterial isolation was as described by Iino *et al.* (2007). NBRC medium 802 containing 1.0% Polypepton (Wako), 0.2% yeast extract (Difco), 0.1% MgSO₄·7H₂O and 1.5% agar (if required) was used for general laboratory cultivation, morphological studies and for determining the optimal growth para-

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Kis4-28^T is AB455532.

A phylogenetic tree derived from 16S rRNA gene sequences of strain Kis4-28^T and its taxonomic neighbours, including many strains in the suborder *Micrococcinae*, is available as a supplementary figure with the online version of this paper.

meters. The medium was adjusted to pH 7.0. The colony morphology was examined after incubation at 28 °C for 3 days. The cell morphology was investigated by examining cells from cultures over a whole growth cycle (up to 5 days) under a light microscope (BX-51, Olympus) and a scanning electron microscope (JSM-6060, JEOL). Cell motility was determined by observing cells suspended in saline solution under a light microscope. Gram staining was performed by using Hucker's modification (Gerhardt *et al.*, 1994). Growth under anaerobic conditions was determined by incubating in an anaerobic chamber with an O₂-absorbing and CO₂-generating agent (Anaero-Pack, Mitsubishi Gas Chemical) and using API OF medium (bioMérieux) according to the manufacturer's instructions. The growth parameters (temperature, pH and NaCl concentration) were determined by measuring the turbidity (610 nm) of 5 ml of the culture medium in test tubes after 1 to 4 days of incubation. The temperature range and the optimum temperature for growth were determined by incubating the cultures at 5, 10, 15, 20, 25, 28, 37, 45 and 60 °C. The pH range and the optimum pH for growth were established by using a medium that was adjusted to pH between 4 and 10 with either 4 M HCl or 5 M KOH and incubated at 28 °C. Tolerance of NaCl (1, 3, 5, 7, 10 and 15 %) was tested at 28 °C. DNase test agar (Difco) was used for the DNase assay. Methyl red and Voges–Proskauer tests were performed using MR–VP medium (Kyokuto Pharmaceutical Industrial) according to the manufacturer's instructions. Phenylalanine deaminase was tested as described by Ewing *et al.* (1957). Other physiological and biochemical tests were performed using API ZYM, API Coryne, API 20E and API 50CH systems (bioMérieux) according to the manufacturer's instructions. Biomass for chemotaxonomic and molecular systematic studies was prepared by growing the strain in shake flasks containing NBRC medium 802 for 24 to 72 h at 28 °C, with shaking.

Strain Kis4-28^T formed yellowish orange, circular, smooth colonies that were approximately 1–2 mm in diameter. Cells in liquid medium were yellowish pink. Growth occurred under both aerobic and anaerobic conditions. The cells were irregular, short, rod-shaped (0.4–0.5 × 1.0–2.0 µm), Gram-positive, non-motile and non-sporulating. Growth occurred at 10–37 °C, and no growth was observed at 5, 45 or 60 °C. The pH range for growth was 6.0–9.0. Optimal growth was noted at 28 °C and pH 7.0. Strain Kis4-28^T exhibited good growth at NaCl concentrations ranging from 0 to 7 % (w/v) and moderate growth at concentrations of 10 %; however, no growth was observed in the presence of 15 % NaCl. The optimal NaCl concentration range for growth was 0–5 %. The results of other physiological and biochemical analyses have been summarized in the species description.

Cell-wall samples were prepared from approximately 1 g wet cells by mechanical disruption with an ultrasonic oscillator and were purified as described by Schleifer & Kandler (1972). The amino acids in cell-wall hydrolysates were analysed using the methods described by Tamura *et al.*

(1994). Amino acid isomers in the cell-wall hydrolysates were determined using the method described by Nozawa *et al.* (2007) with the following modifications. Separation of the 1-fluoro-2,4-dinitrophenyl-5-L-leucinamide and 1-fluoro-2,4-dinitrophenyl-5-D-leucinamide derivatives of amino acids was performed using a model LC-20AB HPLC apparatus (Shimadzu) equipped with a Wakopak wakosil PTC column (Wako). The cellular fatty acids were processed and analysed as methyl esters, using the protocol of the MIDI Sherlock Microbial Identification System (MIS) (Sasser, 1990). Menaquinones were extracted from a 300 mg freeze-dried cell sample and analysed according to the method described by Tamura *et al.* (1994). Cell-wall sugars, polar lipids and the DNA G + C content were also analysed as described by Tamura *et al.* (1994). Mycolic acids were analysed by the method of Minnikin *et al.* (1975), and the acyl type of the muramic acid in the cell-wall was determined by using the method described by Uchida *et al.* (1999).

The purified peptidoglycan of strain Kis4-28^T contained alanine, glutamic acid, lysine and serine (Ser) in a molar ratio of 1.0:2.1:0.9:1.0. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of D-Ala, D-Glu, L-Glu, L-Ser and L-Lys; however, L-Ala was absent. These data indicated that the cell-wall peptidoglycan of strain Kis4-28^T was of the A4α type (Schleifer & Kandler, 1972), with lysine as the diagnostic cell-wall diamino acid, an interpeptide bridge of the peptidoglycan comprising L-Lys–L-Glu, and an L-Ser residue at position 1 of the peptide subunit. The major cellular fatty acids were anteiso-C_{15:0} (45.7 %), C_{16:0} (24.4 %), iso-C_{16:0} (11.5 %) and anteiso-C_{17:0} (10.5 %), while minor components were C_{14:0} (4.1 %) and iso-C_{14:0} (3.8 %). The polar lipids were phosphatidylethanolamine and phosphatidylglycerol. Glycolipids were not detected. MK-8(H₄) was the only menaquinone detected. Galactose was the major cell-wall sugar, while mannose, ribose and xylose were detected as minor components. Strain Kis4-28^T did not contain mycolic acids, and the acyl type of muramic acid was N-acetyl. The DNA G + C content was 70.7 mol%.

PCR amplification of the 16S rRNA gene of strain Kis4-28^T was performed as described by Tamura & Hatano (2001). This gene was directly sequenced using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) and an automated DNA sequencer (ABI PRISM 3730 Genetic Analyzer, Applied Biosystems). The 16S rRNA gene sequence determined in this study was aligned with reference sequences of the genera *Salana*, *Beutenbergia* and *Georgenia* and some related taxa by using the CLUSTAL_X program (Thompson *et al.*, 1997). A phylogenetic tree was reconstructed by using the neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood methods (Felsenstein, 1981). The resultant neighbour-joining tree topology was evaluated by bootstrap analysis based on 1000 replicates (Felsenstein, 1985). The almost-complete 16S rRNA gene sequence (1483 nt) was compared with those from related taxa. Phylogenetic

analysis performed on the basis of the 16S rRNA gene sequence revealed that strain Kis4-28^T formed a clade with the type strains of *Salana multivorans* and *Beutenbergia cavernae* of the suborder *Micrococcineae* (Fig. 1). In the neighbour-joining tree, strain Kis4-28^T joined the phylogenetic lineage of *Salana multivorans* DSM 13521^T at a bootstrap resampling value of 96 %. The similarity values of the 16S rRNA gene sequences were 96.2 and 96.5 % for *Salana multivorans* and *Beutenbergia cavernae*, respectively.

The amino acid composition of the peptidoglycan of strain Kis4-28^T was different from that of the genera *Salana* and *Beutenbergia*. The peptidoglycan of the isolate was of the A4 α type, with L-Lys as the diagnostic cell-wall diamino acid. In contrast, the peptidoglycan of the genus *Salana* is reported to be of the A4 β type, with L-ornithine as the diagnostic diamino acid (von Wintzingerode *et al.*, 2001). Although the peptidoglycan type in species of the genus *Beutenbergia* is also A4 α (Groth *et al.*, 1999), strain Kis4-28^T was different from this genus in that it contained L-Ser at position 1 of the peptide subunit. In addition, strain Kis4-28^T possessed phosphatidylethanolamine as a polar lipid, and contained C_{16:0} as a major cellular fatty acid. These data indicated that the chemotaxonomic character-

istics of this strain were distinct from those of its phylogenetic neighbours (Table 1). Thus, the results of the chemotaxonomic and phylogenetic analyses distinguished strain Kis4-28^T from related genera. Therefore, strain Kis4-28^T represents a novel genus and species, for which the name *Serinibacter salmoneus* gen. nov., sp. nov. is proposed.

The results of phylogenetic analysis of the 16S rRNA gene sequence clearly demonstrated that strain Kis4-28^T and the members of the genera *Beutenbergia* and *Salana* form a monophyletic cluster, and the branch corresponding to this cluster was supported by a bootstrap value of 100 % in the neighbour-joining analysis (Fig. 1). This relationship among these three taxa was also evident in the phylogenetic tree generated by the maximum-likelihood algorithm. Although the genera *Beutenbergia*, *Salana* and *Georgenia* have been placed in the family *Beutenbergiaceae* (Zhi *et al.*, 2009), the genus *Georgenia* forms a monophyletic cluster with the genus *Bogoriella*. The cluster containing the genera *Beutenbergia*, *Salana* and *Serinibacter* and that containing the genera *Georgenia* and *Bogoriella* have their own unique 16S rRNA gene signature nucleotides, when compared with the other families of the suborder *Micrococcineae* (Table 2).

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences of strain Kis4-28^T and its taxonomic neighbours. The tree was constructed by using the neighbour-joining method. The 16S rRNA gene sequence of *Brevibacterium linens* DSM 20425^T (GenBank accession no. X77451) was used as the outgroup (not shown). Numbers at branch nodes are bootstrap percentages (from 1000 replicates); only values of 50 % or above are shown. Dots indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. A larger tree including many strains in the suborder *Micrococcineae* is shown as Supplementary Fig. S1 (available in IJSEM Online). Bar, 0.01 K_{nuc}.

Table 1. Differential phenotypic characteristics of strain Kis4-28^T and the genera *Salana* and *Beutenbergia*

Data for reference genera were taken from von Wintzingerode *et al.* (2001) (*Salana*) and Groth *et al.* (1999) (*Beutenbergia*). The following characteristics apply to all taxa: MK-8(H₄) is the predominant menaquinone; positive for the Gram stain; non-motile and non-sporulating.

Characteristic	Kis4-28 ^T	<i>Salana</i>	<i>Beutenbergia</i>
Cell morphology	Irregular, short rods	Rod, coccoid, club-like	Rod-coccus cycle
Peptidoglycan type	A4 α	A4 β	A4 α
Diamino acid	L-Lys	L-Orn	L-Lys
Position 1*	L-Ser	L-Ser	L-Ala
Major fatty acids (>20 %)	ai-C _{15:0} , C _{16:0}	ai-C _{15:0} , C _{14:0}	i-C _{15:0} , ai-C _{15:0}
Polar lipids†	PE, PG	PG, DPG, PLs	PI, DPG, 3 PLs
DNA G + C content (mol%)	71	75	71

*Amino acid at position 1 of peptide subunit.

†DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLs, unknown phospholipids.

Therefore, on the basis of the phylogenetic analysis and the unique set of 16S rRNA gene signature nucleotides, it is proposed that the genus *Georgenia* is placed in the family *Bogoriellaceae*. The descriptions of the families *Beutenbergiaceae* Zhi *et al.* 2009 and *Bogoriellaceae* Schumann and Stackebrandt 2000 emend. Zhi *et al.* 2009 need to be emended because the unique sets of 16S rRNA gene signature nucleotides reported for these families have been revised. Although the genus *Ruania* was found to be related to the above-mentioned families, as shown in Fig. 1, this genus did not form a reliable clade with any of these families, and the 16S rRNA gene signature nucleotides were also different (Table 2). Hence, the genus *Ruania* does not

belong to either of these families. Further studies are required to elucidate the taxonomic position of this genus.

Description of *Serinibacter* gen. nov.

Serinibacter (Se.ri'ni.bac.ter. N.L. n. *serinum* serine; N.L. masc. n. *bacter* rod; N.L. masc. n. *Serinibacter* a rod with serine in the cell wall).

Cells are irregular, rod-shaped, Gram-positive and non-sporulating. Growth occurs under both aerobic and anaerobic conditions. The cell-wall peptidoglycan is of the A4 α type with an L-Ser residue at position 1 of the peptide subunit. The menaquinone is MK-8(H₄), and the

Table 2. Patterns of selected 16S rRNA gene sequence signature nucleotides of related families and the genus *Ruania* of the suborder *Micrococccineae*

Patterns are indicated for the families *Beutenbergiaceae* (1) (genera *Beutenbergia*, *Salana* and *Serinibacter*), *Bogoriellaceae* (2) (genera *Bogoriella* and *Georgenia*), *Promicromonosporaceae* (3), *Cellulomonadaceae* (4), *Rarobacteraceae* (5) and *Sanguibacteraceae* (6) and the genus *Ruania* (7). K, Guanine or uracil; R, adenine or guanine; S, cytosine or guanine; Y, cytosine or uracil. Residues in lower case letters are present in some but not all strains.

Position	1	2	3	4	5	6	7
144–178	C–G	U–G	U–G	C–G	U–G	c–g	U–G
258–268	G–C	G–C	G–C	G–C	G–C	R–Y	G–C
280	U	C	C	C	C	C	C
293–304	G–U	G–U	G–C	G–C	G–C	G–C	G–U
379–384	C–G	C–G	C–G	C–G	S–S	C–G	C–G
479	G	U	g	G	G	G	G
586–755	C–G	C–G	C–G	C–G	U–G	C–G	C–G
589–650	Y–R	Y–R	U–A	U–A	U–A	U–A	U–A
602–636	C–G	C–G	G–U	S–K	G–U	G–U	G–U
668–738	A–U	A–U	A–U	u–a	A–U	U–A	A–U
1003–1038	G–U	G–C	G–Y	G–C	G–U	G–U	G–U
1027–1034	U–A	C–G	y–a	C–A	U–A	U–A	U–A
1409–1491	A–U	C–G	A–U	A–U	A–U	a–u	C–G
1414–1486	U–A	C–G	C–G	c–g	C–G	C–G	U–G

major polar lipids are phosphatidylethanolamine and phosphatidylglycerol. The fatty acid profile consists of iso- and anteiso- branched and straight-chain fatty acids. The major cell-wall sugar is galactose. Mycolic acids are absent, and the acyl type of muramic acid is *N*-acetyl. Phylogenetically, the genus *Serinibacter* belongs to the family *Beutenbergiaceae* within the suborder *Micrococcineae*. The type species is *Serinibacter salmoneus*.

Description of *Serinibacter salmoneus* sp. nov.

Serinibacter salmoneus (sal.mo'ne.us. N.L. masc. adj. *salmoneus* salmon-coloured, yellowish pink, because the cells grown in liquid culture are yellowish pink).

Shows the following characteristics in addition to those given in the genus description. Cells are 0.4–0.5 × 1.0–2.0 µm and non-motile. Colonies are circular, smooth and yellowish orange, but cells grown in liquid culture are yellowish pink. Catalase-positive, oxidase-negative. The Voges–Proskauer test is positive, while the methyl red test is negative. Growth occurs at NaCl concentrations of 0–10 % (w/v) but not at 15 %, and the optimal NaCl concentration range for growth is 0–5 %. The temperature range for growth is 10–37 °C, and the optimal temperature is 28 °C. The pH range for growth is 6.0–9.0, and the optimal pH is 7.0. Acid is produced from *N*-acetylglucosamine, cellobiose, D-fructose, D-galactose, gentiobiose, D-glucose, glycogen, lactose, maltose, D-mannose, methyl α-D-glucopyranoside, methyl β-D-xylopyranoside, starch, sucrose, trehalose and D-xylose. Esterase lipase (C8), leucine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase, α-glucosidase, pyrazinamidase and pyrrolidonyl arylamidase are present, whereas alkaline phosphatase, lipase (C4), valine arylamidase, cystine arylamidase, trypsin, chymotrypsin, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine- and ornithine decarboxylases and tryptophan- and phenylalanine deaminases are absent. H₂S and indole are not produced. Aesculin is hydrolysed, while gelatin, urea and DNA are not hydrolysed. Nitrate is not reduced. The interpeptide bridge of the cell-wall peptidoglycan is L-Lys–L-Glu. The major cellular fatty acids are anteiso-C_{15:0} and C_{16:0}, followed by iso-C_{16:0} and anteiso-C_{17:0}. The DNA G + C content of the type strain is 70.7 mol%.

The type strain Kis4-28^T (=NBRC 104924^T =DSM 21801^T) was isolated from the intestinal tract of sillago fish (*Sillago japonica*) collected from Kyonan beach on the coast of Tokyo Bay, Japan.

Emended description of the family *Beutenbergiaceae* Zhi *et al.* 2009

The pattern of 16S rRNA gene sequence signatures consists of nucleotides at positions 144:178 (C–G), 280 (U), 293:304 (G–U), 668:738 (A–U), 1003:1038 (G–U), 1027:1034 (U–A) and 1414:1486 (U–A). The family

contains the type genus *Beutenbergia* (Groth *et al.*, 1999) as well as the genera *Salana* (von Wintzingerode *et al.*, 2001) and *Serinibacter*.

Emended description of the family *Bogoriellaceae* Schumann and Stackebrandt 2000, emend. Zhi *et al.* 2009

The pattern of 16S rRNA gene sequence signatures consists of nucleotides at positions 144:178 (U–G), 293:304 (G–U), 479 (U), 602:636 (C–G), 668:738 (A–U), 1003:1038 (G–C), 1027:1034 (C–G) and 1409:1491 (C–G). The family contains the type genus *Bogoriella* (Groth *et al.*, 1997) as well as the genus *Georgenia* (Altenburger *et al.*, 2002).

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