Sanguibacter marinus sp. nov., isolated from coastal sediment

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A Gram-positive, coryneform bacterium, strain 1-19^T, was isolated from coastal sediment from the Eastern China Sea. Phylogenetic analysis based on 16S rRNA gene sequences showed that the organism formed a robust clade with the type species of the genus *Sanguibacter* and displayed less than 97 % gene sequence similarity. Phenotypic characteristics supported the assignment of this organism to the genus *Sanguibacter*. A range of biochemical features distinguished it from all other *Sanguibacter* species with validly published names. On the basis of a polyphasic taxonomical analysis, it is proposed that this bacterium is a novel species of *Sanguibacter*, for which the name *Sanguibacter marinus* sp. nov. is proposed. The type strain is 1-19^T (= CGMCC 1.3457^T = JCM 12547^T).

The genus *Sanguibacter* was proposed by Fernández-Garayzábal *et al.* (1995) and currently contains only three species with validly published names, *Sanguibacter inulinus* (Pascual *et al.*, 1996), *Sanguibacter keddiei* and *Sanguibacter suarezii* (Fernández-Garayzábal *et al.*, 1995). Members of this genus were isolated from the blood of healthy cows. Data on the occurrence of some other *Sanguibacter* strains in marine sediments or subsurface environments, such as oil reservoirs, are available from public databases, e.g. GenBank accession no. AB126692. During an investigation into the bacterial community of coastal sediment from the Eastern China Sea, a novel strain, 1-19^T, was isolated. The present study was designed to clarify the taxonomic position of this strain.

Strain 1-19^T was isolated on plates of Difco marine agar 2216 (Difco) that had been seeded with a coastal sediment suspension and incubated at 30 °C for 5 days. The sediment sample was collected from the Eastern China Sea, Fujian province, China. The isolate was maintained on nutrient agar or trypticase soy agar (TSA; BBL) slants at 4 °C and as a glycerol suspension (20 %, v/v) at –20 °C. Biomass for the chemical and molecular systematic studies was prepared as previously described (Huang *et al.*, 2002) but modified by growing the strain for 3 days.

Cultural and morphological properties were examined by routine observation and with an electron microscope (FEI Quanta) after aerobic growth on TSA for 2–5 days at 30 °C.

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The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of *Sanguibacter marinus* 1-19^T is AJ783958.

The ability of the strain to grow anaerobically was tested using the GENbox anaerobic system (bioMérieux) by incubating the TSA plate in an anaerobic jar. Flagellum staining was performed according to Heimbrook *et al.* (1989). General phenotypic tests were carried out following the procedures of Gordon *et al.* (1974) and Schofield & Schaal (1981). Fermentation of carbohydrates was determined using the API 50 CH kit (bioMérieux) according to the manufacturer's instructions and the reactions were recorded after incubation for 2, 5, 10 and 15 days at 30 °C. Cell-wall peptidoglycan was prepared by extraction with a hot solution of 10 % trichloroacetic acid, followed by treatment with trypsin in phosphate buffer (5 mg trypsin in 10 ml 0.1 M potassium phosphate buffer, pH 7.9) according to the rapid screening procedure described by Schleifer & Kandler (1972). After two washes with distilled water, the pellet was hydrolysed with 4 M HCl at 100 °C for 60 min. Amino acids and peptides in the hydrolysates were separated by two-dimensional ascending TLC on cellulose sheets using two solvent systems. The first direction was developed with 2-propanol/acetate/H₂O (75:10:15) and the second was developed using α -picoline/25 % NH₄OH/H₂O (70:2:28). The developed cellulose sheets were stained by spraying with ninhydrin solution and heating at 70 °C for 5 min. Cellular menaquinones were extracted and purified as described by Collins (1985) and were analysed by HPLC (Wu *et al.*, 1989). Mycolic acids were detected using the acid methanolysis procedure (Minnikin *et al.*, 1975). Cellular fatty acids were extracted, methylated and analysed by GC using the Sherlock MIDI (Microbial Identification) system.

Genomic DNA of strain 1-19^T was extracted according to the method of Marmur (1961). PCR amplification and

sequencing of the 16S rRNA gene were performed as described previously (Zhang *et al.*, 2003). The nucleotide sequence was obtained automatically by using a DNA sequencer (model 377; Applied Biosystems) and software provided by the manufacturer. The 16S rRNA gene sequence of strain 1-19^T was aligned manually against corresponding sequences retrieved from the GenBank database using the CLUSTAL X 1.8 program (Thompson *et al.*, 1997). Phylogenetic trees were inferred by using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) algorithms from the PHYLIP package version 3.5c (Felsenstein, 1993). Evolutionary distance matrices were generated as described by Kimura (1980). The resulting unrooted tree topologies were evaluated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 1000 resamplings. The PHYLIP package (Felsenstein, 1993) was used for all phylogenetic analyses. The DNA G + C content was determined using the thermal denaturation (T_m) method (Marmur & Doty, 1962) with *Escherichia coli* K-12 as the standard.

Comparison of the almost complete 16S rRNA gene sequence of strain 1-19^T (1486 nucleotides) with other sequences found by a BLAST search (Altschul *et al.*, 1990) of the GenBank database showed that strain 1-19^T formed a distinct phyletic line at the periphery of the evolutionary clade occupied by the genus *Sanguibacter* (Fig. 1). The taxonomic integrity of the strain and the three *Sanguibacter* species with validly published names was supported by the results obtained with all three tree-making algorithms and by a 100 % bootstrap value based on the neighbour-joining method. The 16S rRNA gene sequence similarities between strain 1-19^T and its nearest neighbours were 96.9 % (*S. inulinus*), 96.8 % (*S. keddii*) and 96.7 % (*S. suarezii*).

These values fall into the range recorded for many coryneform bacteria at the species level (Behrendt *et al.*, 2002; Fernández-Garayzábal *et al.*, 1995; Osorio *et al.*, 1999; Pascual *et al.*, 1996; Yassin *et al.*, 2002). Similarity values between strain 1-19^T and members of other genera were below 96.0 %.

The chemotaxonomic data for strain 1-19^T are consistent with its assignment to the genus *Sanguibacter*. The amino acid composition and cell-wall peptidoglycan pattern were the same as those of the type species of the genus, *S. keddii*. Cells contained tetrahydrogenated menaquinones with nine isoprene units [MK-9(H₄)] as the predominant isoprenoid quinone. No mycolic acids were detected. The fatty acid profile consisted of anteiso-C_{15:0} (46.7 %), anteiso-C_{15:1A} (17.7 %), C_{16:0} (10.7 %), C_{15:0} (9.3 %), iso-C_{16:0} (3.9 %), C_{14:0} (3.9 %), anteiso-C_{17:0} (2.4 %), anteiso-C_{13:0} (2.3 %), iso-C_{14:0} (1.3 %), C_{17:0} (1.0 %) and iso-C_{15:0} (0.9 %). The assignment of strain 1-19^T to the genus *Sanguibacter* is also supported by other phenotypic characteristics (see species description). However, strain 1-19^T can be easily distinguished from the *Sanguibacter* species with validly published names by a combination of biochemical features (Table 1).

It is evident from the genotypic and phenotypic data that strain 1-19^T forms a distinct centre of taxonomic variation in the genus *Sanguibacter*. Therefore, it is proposed that the organism be classified as *Sanguibacter marinus* sp. nov.

Description of *Sanguibacter marinus* sp. nov.

Sanguibacter marinus (ma.ri'nus. L. masc. adj. *marinus* pertaining to the sea, where the isolate was found).

Cells are Gram-positive, non-endospore-forming, short, irregular, motile rods with sparse peritrichous flagella.

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing the phylogenetic position of strain 1-19^T. Asterisks indicate branches that were also recovered using the least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Kluge & Farris, 1969) algorithms. The numbers at the nodes indicate the percentages of bootstrap support based on a neighbour-joining analysis of 1000 resampled datasets; only values over 50 % are given. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differentiation of the species of the genus *Sanguibacter* based on phenotypic characteristics

Data for reference species from Fernández-Garayzábal *et al.* (1995) and Pascual *et al.* (1996). +, Positive; –, negative; V, variable.

Characteristic	<i>S. marinus</i>	<i>S. inulinus</i>	<i>S. keddieii</i>	<i>S. suarezii</i>
Nitrate reduction	+	V*	–	V
Acid production from:				
<i>N</i> -Acetylglucosamine	–	+	+	–
Amygdalin	–	+	+	+*
Gentiobiose	–	+	+	+
Gluconate	–	+	V	–
Glycerol	–	+	+	+
Inulin	–	+	–	–
5-Ketogluconate	–	+	–	–
D-Lyxose	–	+	+	+
D-Mannitol	–	V	–	–
D-Melibiose	–	+	+	+
Methyl α -D-glucoside	+*	+	+	–
Methyl α -D-mannoside	–	+	+	–
Methyl β -D-xyloside	–	V	+	+
L-Rhamnose	–	+	+	+
D-Ribose	–	+	+	+
D-Sorbitol	–	–	+	–
D-Turanose	–	+	+	+

*Weak or delayed reaction.

Colonies are circular, convex with entire edges and pale yellow. Diffusible pigments are not produced. Growth occurs under aerobic and anaerobic conditions on TSA medium. Catalase-positive and oxidase-negative. Growth occurs at 15–37 °C (optimum 25–30 °C), pH 5.5–9.0 and 0–7 % (w/v) NaCl. No growth at 42 °C and 10 % NaCl. Citrate is not utilized. Aesculin, gelatin and starch are hydrolysed, but casein, cellulose and Tween 80 are not. Methyl red and indole tests are positive, but Voges–Proskauer test is negative. Acid is produced from L-arabinose, arbutin, D-cellobiose, D-fructose, D-galactose, D-glucose, glycogen, D-lactose, D-maltose, D-mannose, salicin, D-sucrose, D-trehalose and D-xylose, but not from D-adonitol, D-arabinose, DL-arabitol, dulcitol, *meso*-erythritol, DL-fucose, inositol, 2-ketogluconate, D-melezitose, D-raffinose, L-sorbose, D-tagatose, xylitol or L-xylose. Additional biochemical characteristics are given in Table 1. The cell-wall peptidoglycan type is the same as that of *S. keddieii*. The menaquinones are MK-9(H₄) and MK-9(H₆) (peak area ratio, 10.4:1). The cellular fatty acid profile consists mainly of anteiso methyl-branched type anteiso-C₁₅ and straight-chain saturated components. The DNA G + C content is 73.4 mol% (*T_m* method).

The type strain, 1-19^T (= CGMCC 1.3457^T = JCM 12547^T), was isolated from coastal sediment collected in the Fujian province of China.

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