Sanguibacter antarcticus sp. nov., isolated from Antarctic sea sand

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A Gram-positive, yellow-pigmented bacterium, strain KOPRI 21702^T, was isolated from sea sand on King George Island, Antarctica. Cells were irregular rods with peritrichous flagella; their optimum growth temperature was 23–26 °C. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the Antarctic isolate formed a distinct phyletic line in a clade of the genus *Sanguibacter* and showed highest sequence similarity (97.7 %) to the type strain of *Sanguibacter keddieii*. The major isoprenoid quinone, predominant cellular fatty acids and DNA G+C content were consistent with placement of the Antarctic isolate in the genus *Sanguibacter*. Phylogenetic analysis and differences in physiological and biochemical characteristics between strain KOPRI 21702^T and the four recognized *Sanguibacter* species indicate that the isolate represents a novel species of this genus. The name *Sanguibacter antarcticus* sp. nov. (type strain KOPRI 21702^T =KCTC 13143^T =JCM 14623^T =DSM 18966^T) is proposed for this isolate.

The genus *Sanguibacter* was established by Fernández-Garayzábal *et al.* (1995) for isolates from blood samples of dairy cows and, at present, contains four species with validly published names: *Sanguibacter suarezii*, *Sanguibacter keddieii* and *Sanguibacter inulinus*, isolated from the blood of cows (Fernández-Garayzábal *et al.*, 1995; Pascual *et al.*, 1996), and *Sanguibacter marinus*, from a marine environment (Huang *et al.*, 2005).

Strain KOPRI 21702^T was isolated from a sea sand sample collected on the Weaver Peninsula (62° 13′ 45″ S 58° 47′ 15″ W) on King George Island, Antarctica. Isolation was carried out using ZoBell agar (ZoBell, 1946) supplemented with 0.4 % colloidal chitin at 20 °C. The isolate was maintained routinely on ZoBell agar at 20 °C and preserved as suspensions of cells in glycerol (10 %, v/v) at −80 °C.

The 16S rRNA gene was enzymically amplified from a single colony with universal primers described by Lane (1991). The sequence was aligned manually with those of type strains of *Sanguibacter* species obtained from GenBank. Secondary structural information implemented in the jPHYDIT program (Jeon *et al.*, 2005) was used for accurate alignment. Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods using the program PAUP (Swofford, 2002). An evolutionary distance matrix for the neighbour-joining method was generated according to Kimura's two-parameter model (Kimura, 1980). The maximum-parsimony tree was constructed using the exhaustive search option. The confidence level of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985) using 1000 replications. An almost-complete 16S rRNA gene sequence of strain KOPRI 21702^T was obtained (1468 bp). Preliminary comparisons with 16S rRNA gene sequences from GenBank revealed that the isolate showed highest sequence similarity to the type strains of the four recognized *Sanguibacter* species, i.e. *S. keddieii* ATCC 51767^T (97.7 % similarity), *S. suarezii* ST-26^T (97.3 %), *S. inulinus* ST-50^T (97.3 %) and *S. marinus* 1-19^T (96.8 %). Phylogenetic analysis including members of *Sanguibacter* and related genera showed that the strain formed a distinct lineage within the genus *Sanguibacter* monophyletic group supported with high bootstrap values in all tree-making methods (data not presented). A reduced parsimony tree is shown in Fig. 1.

Chemotaxonomic characteristics were determined in cells grown at 30 °C on TSA medium (Difco). Isoprenoid quinones were extracted from 2-day-old cells according to the method of Minnikin *et al.* (1984); HPLC analysis (Collins, 1985) revealed MK-9(H_4) as the predominant isoprenologue. Fatty acid methyl ester analysis was performed by GC (model 6890A; Hewlett-Packard) according to the Microbial Identification (MIDI) system

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KOPRI 21702^T is EF211071.

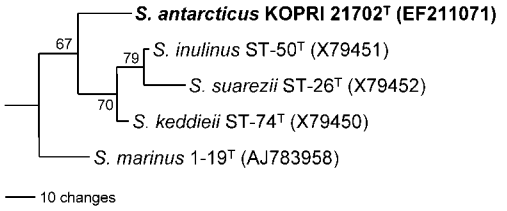

Fig. 1. Phylogenetic position of strain KOPRI 21702^T within the genus *Sanguibacter* based on 16S rRNA gene sequences. The tree was reconstructed by an exhaustive search with the parsimony criterion. Bootstrap support (%) is given at nodes (only values >50 % are shown). *Micrococcus luteus* DSM 20030^T was used as an outgroup (not shown).

using 1-day-old cells. The fatty acid profile consisted of anteiso-$C_{15:0}$ (50.5 %), $C_{16:0}$ (9.6 %), anteiso-$C_{13:0}$ (7.6 %), iso-$C_{16:0}$ (7.5 %), anteiso-$C_{17:0}$ (6.4 %), anteiso-$C_{15:1}$ A (4.5 %), $C_{14:0}$ (4.3 %), iso-$C_{17:1}$ I/anteiso-$C_{17:1}$ B (2.1 %), iso-$C_{14:0}$ (1.9 %), $C_{17:0}$ (1.5 %) and iso-$C_{15:0}$ (1.1 %); small amounts (less than 1 %) of other fatty acids were also present. The DNA G+C content was 69.5 mol%, as determined by analysis of deoxyribonucleosides (Mesbah *et al.*, 1989) using HPLC equipped with a C18 reversed-phase column (Supelco). The above characteristics of strain KOPRI 21702^T are consistent with its assignment to the genus *Sanguibacter*.

The temperature range for growth was determined in a temperature-gradient incubator (TVS126MA; Advantec) using tryptic soy broth (Difco) in the range 5–50 °C. Optimal growth was obtained at 23–26 °C and the maximum temperature for growth was 30 °C. Growth at different pH (between pH 4 and 10 at intervals of 1 pH unit) and NaCl concentrations [between 0 and 10 % (w/v) at intervals of 2 or 3 %] was determined using tryptic soy broth (Difco). Growth in anaerobic conditions was examined in the anaerobic chamber with an atmosphere of 80 % nitrogen, 10 % carbon dioxide and 10 % hydrogen at 30 °C for up to 5 days using thioglycollate medium (Sigma). Enzyme activities, assimilation and fermentation of sole carbon sources, nitrate reduction and indole production were determined using the API 20E, API 20NE and API 50CHB systems (bioMérieux). Morphological examinations of cells of strain KOPRI 21702^T were performed by differential interference contrast microscopy and scanning and transmission electron microscopy of cells grown on TSA (Difco) at 30 °C for 2 days. Cellular motility was tested on motility medium (0.3 % beef extract, 1 % peptone, 0.5 % NaCl, 0.4 % agar). Detailed results of these experiments are given in the species description. Morphological and physiological characteristics that differentiate strain KOPRI 21702^T from the other species of the genus *Sanguibacter* are given in Table 1.

The strain can be readily differentiated from other members of the genus *Sanguibacter* by its maximal growth temperature, the ability to reduce nitrate, the inability to hydrolyse gelatin and acid production from various carbon sources, as presented in Table 1. Therefore, it is concluded that strain KOPRI 21702^T should be assigned to the genus *Sanguibacter* as the type strain of a novel species. The name *Sanguibacter antarcticus* sp. nov. is proposed for the Antarctic isolate.

Table 1. Characteristics that differentiate strain KOPRI 21702^T from related *Sanguibacter* species

Strains: 1, *S. antarcticus* sp. nov. KOPRI 21702^T; 2, *S. inulinus* ST-50^T; 3, *S. keddieii* ST-74^T; 4, *S. marinus* 1-19^T; 5, *S. suarezii* ST-26^T. +, Positive; −, negative; ND, not determined; V, variable; W, weakly positive. Data are taken from this study and from Fernández-Garayzábal *et al.* (1995), Pascual *et al.* (1996) and Huang *et al.* (2005).

Characteristic	1	2	3	4	5
Growth at 35 °C on agar	−	+	+	+	+
Nitrate reduction	+	V	−	+	V
Gelatin hydrolysis	−	ND	+	+	−
Acid production from:					
N-Acetylglucosamine	−	+	+	−	−
Amygdalin	−	+	+	−	W
Arbutin	−	+	+	+	+
D-Galactose	−	+	+	+	+
Gentiobiose	+	+	+	−	+
Gluconate	−	+	V	−	−
Glycerol	−	+	+	−	+
Inulin	−	+	−	−	−
5-Ketogluconate	−	+	−	−	−
D-Lactose	−	+	+	+	+
D-Lyxose	−	+	+	−	+
D-Mannitol	+	V	−	−	−
D-Mannose	−	+	+	+	+
Melibiose	−	+	+	−	+
Methyl α-D-glucoside	−	+	+	W	−
Methyl α-D-mannoside	−	+	+	−	−
Methyl β-D-xyloside	−	V	+	−	+
D-Raffinose	−	+	+	−	V
L-Rhamnose	−	+	+	−	+
D-Ribose	+	+	+	−	+
Salicin	−	+	+	+	+
D-Sorbitol	−	−	+	−	−
Turanose	+	+	+	−	+

Description of *Sanguibacter antarcticus* sp. nov.

Sanguibacter antarcticus (an.tarc′ti.cus. L. masc. adj. *antarcticus* southern, pertaining to the Antarctic, the geographical origin of the type strain).

Gram-positive, catalase-positive, oxidase-negative, facultatively anaerobic and mesophilic. Cells are irregular and motile rods (0.6–45 × 0.4–0.6 μm) with sparse peritrichous flagella. Colonies are yellow, circular and convex with entire edges. Diffusible pigments are not produced. Growth occurs under aerobic and anaerobic conditions. Growth occurs at pH 4–9 (optimum pH 5–6) and with 0–7 % NaCl

(optimum 2–5 %). Grows at 4–30 °C (optimum 23–26 °C). Acid is produced from L-arabinose, cellobiose, aesculin, fructose, gentiobiose, glucose, glycogen, maltose, mannitol, ribose, starch, sucrose, trehalose, turanose and D-xylose (API 50CHB). Acid is not produced from *N*-acetylglucosamine, adonitol, amygdalin, D-arabinose, D-arabitol, L-arabitol, arbutin, dulcitol, erythritol, D-fucose, L-fucose, galactose, gluconate, glycerol, inositol, inulin, 2-ketogluconate, 5-ketogluconate, lactose, D-lyxose, mannose, melibiose, melezitose, methyl α-D-glucoside, methyl α-D-mannoside, methyl β-D-xyloside, raffinose, rhamnose, salicin, sorbitol, sorbose, D-tagatose, xylitol or L-xylose (API 50CHB). Citrate is utilized and acetoin is produced weakly, but negative for arginine dihydrolase, cytochrome oxidase, β-galactosidase, gelatinase, H_2S production, indole production, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase and urease (API 20E). Nitrate is reduced to nitrite and nitrogen (API 20NE). Major isoprenoid quinone is MK-9(H_4). Predominant cellular fatty acids are anteiso-$C_{15:0}$ (50.5 %), $C_{16:0}$ (9.6 %), anteiso-$C_{13:0}$ (7.6 %) and iso-$C_{16:0}$ (7.5 %).

The type strain is KOPRI 21702^T (=KCTC 13143^T =JCM 14623^T =DSM 18966^T), isolated from a sea sand sample from King George Island, Antarctica. The genomic DNA G+C content of the type strain is 69.5 mol%.

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