Sanguibacter soli sp. nov., isolated from soil of a ginseng field

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A Gram-positive, non-spore-forming, rod-shaped, motile bacterium, designated strain DCY22T, was isolated from soil of a ginseng field in South Korea and characterized in order to determine its taxonomic position. 16S rRNA gene sequence analysis revealed that strain DCY22T belonged within the family Sanguibacteraceae, and highest levels of sequence similarity were found with Sanguibacter marinus 1-19T (96.8 %), Sanguibacter suarezii ST-26T (96.0 %), Sanguibacter inulinus ST-50T (95.9 %), Sanguibacter keddii ST-74T (95.5 %), Terrabacter terrae PPLB1T (94.0 %) and Terrabacter tumescens DSM 20308T (93.8 %). Chemotaxonomic investigations revealed that strain DCY22T possessed menaquinone MK-9, a common feature of members of the genus Sanguibacter. Predominant fatty acids were unknown ECL 13.961 (45.81 %), 17:0 anteiso (23.46 %), 18:0 iso (15.42 %) and unknown ECL 14.966 (8.70 %). The results of physiological and biochemical tests clearly demonstrated that strain DCY22T represents a novel species of the genus Sanguibacter, for which the name Sanguibacter soli sp. nov. is proposed. The type strain is DCY22T (=KCTC 13155T=JCM 14841T).

The family Sanguibacteraceae was proposed by Stackebrandt & Schumann (2000) with Sanguibacter as the type genus. Sanguibacter remains the only described genus within the family. The genus Sanguibacter was proposed by Fernández-Garayzabal et al. (1995) and at the time of writing comprises four recognized species, namely Sanguibacter inulinus (Pascual et al., 1996), Sanguibacter keddii (Fernández-Garayzabal et al., 1995), Sanguibacter marinus (Huang et al., 2005) and Sanguibacter suarezii (Fernández-Garayzabal et al., 1995).

In a series of studies, we have attempted to isolate microorganisms from soil in order to investigate the community structure based on a culture-dependent method. In the present study, one strain, designated DCY22T, was isolated from soil in a ginseng field and was characterized based on a polyphasic approach, including 16S rRNA gene sequence analysis, together with investigations of genomic relatedness and chemotaxonomic and phenotypic properties. The results indicate that strain DCY22T represents a novel species of the genus Sanguibacter.

Strain DCY22T was isolated from surface soil of an agricultural field where ginseng was planted. One gram of the soil was immersed in 50 ml saline solution, vortexed and serially diluted and a 100 µl aliquot was inoculated onto one-tenth-strength R2A agar (Difco). The purified colonies were tentatively identified by using analysis of the partial 16S rRNA gene sequence. Cell morphology and motility were observed with a Nikon light microscope (×1000 magnification) after incubation on diluted Luria–Bertani (LB) agar (0.5 % agar) for 1 day. Gram reactions were conducted according to the non-staining method as described by Buck (1982). Oxidase activity was evaluated via the oxidation of 1 % p-aminodimethylaniline oxalate. Catalase activity was determined by measurements of bubble production after the application of 3 % (v/v) hydrogen peroxide solution. Growth at various temperatures (4, 15, 25, 30, 37 and 42 °C) was assessed on R2A agar, and growth at different pH values (pH 5.0–11.0 at intervals of 0.5 pH units) was assessed in R2A broth. Growth on nutrient agar, LB agar and trypticase soy agar (TSA) was also evaluated at 30 °C. The API 20NE, API ID32 GN, API 50CH and API ZYM microtest systems were employed in these tests following the recommendations of the manufacturer (bioMérieux).
Isoprenoid quinones were extracted with chloroform/methanol (2:1, v/v), purified by TLC and subsequently analysed by HPLC, as described by Collins & Jones (1981) and Shin et al. (1996). For fatty acid methyl ester analysis, the strain was allowed to grow on TSA for 48 h at 30 °C, and then two loops of well-grown cells were harvested. Fatty acid methyl esters were prepared, separated and identified by using the Sherlock Microbial Identification System (MIDI, Inc.; Sasser, 1990).

For determination of the G+C content, genomic DNA was extracted and purified with the Qiagen Genomic-tip system 100/G and was then enzymically degraded into nucleosides. The nucleosides were analysed by using HPLC as described by Tamaoka & Komagata (1984) and Mesbah et al. (1989).

The 16S rRNA gene was amplified from chromosomal DNA by using the universal bacterial primer set fD1 and rP1 (Weisburg et al., 1991), and the purified PCR product was sequenced by Genetec (Daejeon, Korea) (Kim et al., 2005). The full 16S rRNA gene sequence was compiled with SeqMan software (DNASTAR). The 16S rRNA gene sequences of related taxa were obtained from GenBank and edited by using the BioEdit program (Hall, 1999). Multiple alignments were performed with the CLUSTAL_X program (Thompson et al., 1997). Evolutionary distances were calculated according to Kimura’s two-parameter model (Kimura, 1983). Phylogenetic trees were constructed via the neighbour-joining method (Saitou & Nei, 1987) and maximum-parsimony method in the MEGA 2 program (Kumar et al., 2001). Bootstrap analysis with 1000 replicates was carried out for the tree construction to evaluate the significance of the branching order (Kumar et al., 2001).
replicates was also conducted in order to obtain confidence levels for the branches (Felsenstein, 1985). The closest relatives of strain DCY22\(^T\), namely the type strains of all recognized Sanguibacter species, and the type species of genera representing all families in the suborder Micrococcineae were included in the phylogenetic tree.

Strain DCY22\(^T\) was cultured on R2A agar (Difco) at 30 °C, yielding yellow, circular colonies. Strain DCY22\(^T\) was found to be an aerobic, Gram-positive, motile, rod-shaped bacterium that was able to grow at 25–42 °C but not at 4 °C. Growth was observed at pH 5–9. The physiological characteristics of strain DCY22\(^T\) are summarized in the species description below, and a comparison of differential characteristics with the type strains of recognized Sanguibacter species is given in Table 1.

The cellular fatty acid profile of strain DCY22\(^T\) is presented in Supplementary Table S1 (available in IJSEM Online). The major cellular fatty acids found in strain DCY22\(^T\) were unknown ECL 13.961 (45.81 %), 17 : 0 anteiso (23.46 %), 18 : 0 iso (15.42 %), unknown ECL 14.966 (8.70 %), 12 : 0 anteiso (3.35 %) and 18 : 3 \(\alpha\),\(\beta\),\(\delta\)-glucosamine (2.63 %). Strain DCY22\(^T\) lacked 15 : 0 anteiso and 16 : 0, both of which are common in recognized species of the genus Sanguibacter and in Terrabacter terrae and Terrabacter tumescens. The fatty acids 17 : 0 anteiso, 18 : 0 iso, unknown ECL 13.961 and ECL 14.966 in DCY22\(^T\) are not major components of the profiles of recognized Sanguibacter and Terrabacter species.

Strain DCY22\(^T\) contained a menaquinone with nine isoprene units (MK-9) as the predominant isoprenoid quinone and a smaller amount of MK-8. MK-9 is commonly found in recognized Sanguibacter species (Pascual et al., 1996; Huang et al., 2005).

The 16S rRNA gene sequence of strain DCY22\(^T\) was found to be a continuous stretch of 1395 nt. Based on preliminary data, strain DCY22\(^T\) was determined to belong to the class Actinobacteria, suborder Micrococcineae, family Sanguibacteraceae. Highest levels of 16S rRNA gene sequence similarity were found with S. marinus 1-19\(^T\) (96.8 %), S. suarezii ST-26\(^T\) (96.0 %), S. inulinus ST-50\(^T\) (95.9 %), S. keddiei ST-74\(^T\) (95.5 %), T. terrae PPLB\(^T\) (94.0 %) and T. tumescens DSM 20308\(^T\) (93.8 %). In the neighbour-joining phylogenetic tree (Fig. 1), strain DCY22\(^T\) clearly fell within the Sanguibacter lineage. A maximum-parsimony tree is available as Supplementary Fig. S1.

The G+C content of the genomic DNA of strain DCY22\(^T\) was 69.8 mol%, a value similar to that reported for recognized Sanguibacter species (69.5–73.4 mol%). On the basis of phenotypic, chemotaxonomic and phylogenetic data, we conclude that strain DCY22\(^T\) represents a novel species of the genus Sanguibacter, for which the name Sanguibacter soli sp. nov. is proposed.

**Description of Sanguibacter soli sp. nov.**

*Sanguibacter soli* (so’li. L. neut. gen. n. soli of soil, the source of the type strain).

Cells are Gram-positive, aerobic, motile rods when grown on R2A agar (Difco) at 30 °C for 5 days. Colonies grown on R2A agar for 5 days are yellow. Optimal growth temperature is 37 °C. Grows at pH 5–9. Oxidase- and catalase-positive. Acid is produced from L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, glycerol, lactose, maltose, maltose, methyl \(\alpha\),\(\beta\),\(\delta\)-glucosamine, starch, sucrose, trehalose, turanose, D-xylose, glycerol, N-acetyl-glucosamine, arbutin, aesculin and salicin, but not from...
gluconate, 2-ketogluconate, 5-ketogluconate, D-arabinose, D- or L-fucose, D-lyxose, melezitose, melibiose, methyl α-D-mannoside, L-rhamnose, raffinose, ribose, L-sorbose, D-tagatose, L-xylitol, D-adenitol (ribitol), D-arabitol, L-arabitol, dulcitol (galactitol), erythritol, inositol, mannoitol, sorbitol, xylitol, amygdalin or inulin. Positive for N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, α-chymotrypsin, cystine arylamidase, esterase (C4), esterase (C8), β-galactosidase, α-glucosidase (aesculin hydrolysis), β-glucuronidase, leucine arylamidase, lipase (C14), α-mannosidase, naphthol-AS-BI-phosphohydrolase, trypsin and valine arylamidase. Negative for arginine dihydrolase, α-fucosidase, α-glactosidase, protease (gelatin hydrolysis) and urease. Assimilates acetate, L-arabinose, D-glucose, maltose, D-mannose, sucrose, glycogen and salicin, but not 3-hydroxybenzoate, 4-hydroxybenzoate, DL-3-hydroxybutyrate, 4-hydroxybenzoate, 2-ketogluconate, 5-ketogluconate, adipate, caprate, citrate, gluconate, itaconate, L-lactate, L-malate, malonate, phenyl acetate, propionate, suberate, n-valerate, L-fucose, melibiose, L-rhamnose, D-ribose, myo-inositol, D-mannitol, D-sorbitol, N-acetyl-D-glucosamine, L-alanine, L-histidine, L-proline or L-serine. The DNA G+C content of the type strain is 69.8 mol%, as determined by HPLC. The predominant quinone is MK-9(H4). Major cellular fatty acids include unknown ECL 13.961 (45.81 %), 17:0 anteiso (23.46 %), 18:0 iso (15.42 %), unknown ECL 14.966 (8.70 %), 12:0 anteiso (3.35 %) and 18:3 ω6,9,12c (2.63 %).

The type strain, DCY225T (=KCTC 13155T =JCM 14841T), was isolated from soil of a ginseng field in South Korea.

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References


