Skermanella stibiiresistens sp. nov., a highly antimony-resistant bacterium isolated from coal-mining soil, and emended description of the genus Skermanella

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A Gram-negative, aerobic, motile, rod-shaped, antimony-resistant bacterium, designated strain SB22<sup>T</sup>, was isolated from soil of Jixi coal mine, China. The major cellular fatty acids (>5%) were C<sub>18:1</sub>ω7c (63.5%), summed feature 2 (C<sub>14:0</sub> 3-OH and/or iso-C<sub>16:1</sub> i, 10.8%) and C<sub>16:0</sub> (9.9%). The major polar lipids were diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and an unknown aminolipid. The genomic DNA G+C content was 69.6 mol% and Q-10 was the major respiratory quinone. Phylogenetic analyses based on 16S rRNA gene sequences indicated that strain SB22<sup>T</sup> was most closely related to Skermanella aerolata 5416T=32<sup>T</sup> (97.3%), Skermanella parooensis ACM 2042<sup>T</sup> (95.8%) and Skermanella xinjiangensis 10-1-101<sup>T</sup> (92.9%). The DNA–DNA hybridization value between strain SB22<sup>T</sup> and S. aerolata KACC 11604<sup>T</sup> (=5416T=32<sup>T</sup>) was 43.3%. On the basis of phenotypic, chemotaxonomic and phylogenetic characteristics of strain SB22<sup>T</sup> and related species, it is considered that the isolate represents a novel species of the genus Skermanella, for which the name Skermanella stibiiresistens sp. nov. is proposed. The type strain is SB22<sup>T</sup> (=CGMCC 1.10751<sup>T</sup>=KCTC 23364<sup>T</sup>). An emended description of the genus Skermanella is provided.

The family Rhodospirillaceae (Pfennig & Trüper, 1971), belonging to the Alphaproteobacteria, contains 27 genera (http://www.bacterio.cict.fr/classifgenerafamilies.html#Rhodospirillaceae) including several genera that have been established in recent years, i.e. Thalassobaculum (Zhang et al., 2008), Nissaea (Urios et al., 2008), Fodinicurvata (Wang et al., 2009), Marispirillum (Lai et al., 2009a), Oceanibaculum (Lai et al., 2009b), Pelagibius (Choi et al., 2009), Tistlia (Diaz-Cárdenas et al., 2010), Dongia (Liu et al., 2010) and Desertiibacter (Liu et al., 2011). The genus Skermanella of the family Rhodospirillaceae was proposed by Sly & Stackebrandt (1999) based on studies of Skermanella parooensis ACM 2042<sup>T</sup>. This type species was originally proposed as Conglomeromonas largomobilis subsp. parooensis by Skerman et al. (1983). Later, it was transferred to the genus Skermanella on the basis of the phylogenetic evidence and phenotypic characteristics (Ben Dekhil et al., 1997; Sly & Stackebrandt, 1999). The genus Skermanella currently comprises three species with validly published names, Skermanella parooensis (Sly & Stackebrandt, 1999), Skermanella aerolata (Weon et al., 2007) and Skermanella xinjiangensis (An et al., 2009), which were isolated from fresh water, air and sandy soil, respectively. The major respiratory quinone in both S. aerolata and S. xinjiangensis was Q-10 (Weon et al., 2007; An et al., 2009). As yet, no polar lipid data have been reported for members of this genus. Species of this genus have been characterized as Gram-negative, motile, strictly aerobic, unable to fix nitrogen, containing C<sub>18:1</sub>ω7c and C<sub>16:0</sub> as the major cellular fatty acids, and possessing a high DNA G+C content (65.0–68.8 mol%) (Sly & Stackebrandt, 1999; Weon et al., 2007; An et al., 2009).

Antimony (Sb) is a metalloid belonging to Group 15 of the Periodic Table that is similar to arsenic. It is a widely distributed trace element of toxicological interest found in the environment and mainly present as Sb(III) and Sb(V) (Shotyk et al., 2005). Antimony is known to cause adverse health effects on humans and animals (Ye et al., 2010), but can be used for the treatment of tropical protozoan diseases, such as leishmaniasis (Berman, 2003; Vásquez et al., 2006). Antimony is generally highly toxic to micro-organisms; thus, identification of Sb-resistant bacteria is fundamentally important for an understanding of microbial antimony metabolism and for applications in bioremediation.

In this study, bacterial strains were isolated from soil collected from Jixi coal mine (45° 18’ N 130° 57’ E) of Jixi City, Heilongjiang Province, north-east China. The soil...
texture was sandy type with a pH of 7.2 and total As, Sb, Fe and Cu concentrations were 0.04, 0.01, 18.0 and 0.09 g kg⁻¹, respectively (determined by atomic absorption spectrometry). Total C, N, P, S and NO₃ concentrations were 303.0, 3.8, 0.6, 0.2 and 0.04 g kg⁻¹, respectively. Sb-resistant bacteria were isolated using CDM medium (l⁻¹: MgSO₄·7H₂O, 2.0 g; NH₄Cl, 1.0 g; Na₂SO₄, 1.0 g; K₂HPO₄, 0.013 g; CaCl₂·2H₂O, 0.067 g; sodium lactate, 5.0 g; FeSO₄·7H₂O, 0.033 g; NaHCO₃, 0.798 g; and 15.0 g agar, pH 7.2) (Weeger et al., 1999) containing 0.1 mM C₈H₄K₂O₁₂Sb₂·3H₂O. About 10⁴ c.f.u. (g soil)⁻¹ were obtained and a total of 14 different Sb-resistant bacterial strains were isolated. Strain SB22T was chosen for this study due to its potential novelty and high resistance to Sb.

For analyses of morphological, physiological and biochemical characteristics, strain SB22T and three reference strains, S. aerolata KACC 11604T, S. parooensis DSM 9527T and S. xinjiangensis CCTCC AB 207153T were cultured on R2A agar (Difco) unless otherwise stated. Motility tests were performed using a stab cultivation method in R2A medium containing 0.3 % agar. Cell morphologies were observed by light and scanning electron microscopics. Gram staining was determined using the method described by Dussault (1955). Growth at different temperatures (4, 20, 28, 32, 37 and 42 °C) was assessed after 7 days incubation on R2A plates. The pH range (4–10) for growth was determined at 28 °C for 7 days in R2A broth. Salt tolerance was tested in R2A broth supplemented with 0–7 % (w/v) NaCl after 7 days incubation. Growth under anaerobic conditions was determined by incubation in an anaerobic chamber (Mitsubishi Gas Chemical) at 28 °C for 15 days on R2A plates.

Hydrolysis of casein, gelatin, starch, Tween 40, Tween 80, DNA, tyrosine and urea was performed as described by Cowan & Steel (1965). Nitrate reduction was tested by the method described by Lanyi (1987). Methyl red and Voges-Proskauer tests, and determination of H₂S and indole production were performed as recommended by Smibert & Krieg (1994). Catalase activity was determined by assessing bubble production in 3 % (v/v) H₂O₂, and oxidase activity was determined using 1 % (w/v) tetramethyl p-phenylenediamine. For acid production from carbohydrates, phenol red broth (Rhoades et al., 1989) was used and determined as described by Hinz et al. (1998). The nitrogen-fixing ability of strain SB22T was tested in nitrogen-free malate medium using the acetylene reduction assay (Eckert et al., 2001). Antibiotic-susceptibility tests were performed by spreading bacterial suspensions on R2A plates, then applying filter-paper discs containing different concentrations of antibiotics and incubating at 28 °C for 2 days. Susceptibility was scored as positive at zone diameters above 10 mm (Hangzhou Microbial Reagent). Enzyme activities, other biochemical characteristics and utilization of carbohydrates as sole carbon sources were determined using API ZYM, API 20 NE and API ID 32 GN kits, respectively, according to the manufacturer’s (bioMérieux) instructions.

The MIC, defined as the lowest Sb(III) concentration that completely inhibits the growth of each strain, was determined as described by Lim & Cooksey (1993) for SB22T and the three reference strains. Triplicate samples of each single bacterial colony were grown overnight at 28 °C with shaking at 160 r.p.m.; then, 2 % original culture was inoculated into 5 ml R2A broth and with serial concentrations of C₈H₄K₂O₁₂Sb₂·3H₂O. Growth of each strain was determined (by OD₆00) after incubation at 28 °C with shaking at 160 r.p.m. for 7 days.

A nearly full-length 16S rRNA gene sequence was identified as described by Fan et al. (2008), and compared with sequences available in NCBI (http://blast.ncbi.nlm.nih.gov/) and EzTaxon server 2.1 (http://147.47.212.35:8080/) (Chun et al., 2007). Multiple alignments were performed with the program CLUSTAL_X (Thompson et al., 1997). Phylogenetic analyses were carried out using MEGA4.0 (Tamura et al., 2007) and the PHYML online web server (Guindon et al., 2005). Neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Kluge & Farris, 1969) trees were reconstructed using MEGA4.0 (Tamura et al., 2007). A maximum-likelihood tree (Felsenstein, 1981) was reconstructed using the PHYML online web server (Guindon et al., 2005) and viewed with MEGA4.0. In each case, bootstrap values were calculated based on 1000 replications.

DNA–DNA hybridization analysis between strain SB22T and S. aerolata KACC 11604T was performed by the thermal denaturation and renaturation method (Huß et al., 1983). The DNA G+C content was determined by HPLC according to the method of Tamaoka & Komagata (1984). Respiratory quinone analysis of strain SB22T and the three reference strains was performed by HPLC as described by Xie & Yokota (2003). For whole-cell fatty acid analysis, strain SB22T and the three reference strains were each grown on R2A broth and biomass was collected in the exponential phase and analysed by GC (Hewlett Packard 6890) according to the instructions of the Sherlock Microbial Identification System (MIDI Sherlock version 4.5, MIDI database TSBA40 4.10) (Kroppenstedt, 1985; Sasser, 1990). Polar lipids of strain SB22T and the three reference strains were analysed under the same experimental conditions by two-dimensional TLC as described by Tindall (1990).

Detailed results of the polyphasic taxonomic characterization of strain SB22T are given in the species description. A scanning electron micrograph showing the general morphology of strain SB22T is shown in Fig. S1 (available in IJSEM Online). The main differential phenotypic characteristics of strain SB22T and the three reference strains are shown in Table 1. Strain SB22T showed characteristics that were typical of members of the genus Skermanella, but there were some clear differences, such as the ability to utilize salicin, l-arabinose, mannitol and propionate (Table 1). Moreover, the MIC for Sb(III) of strain SB22T was 4.0 mM, which was much higher than that for the three reference strains (MICs <0.05 mM). Differences in the Sb-resistance levels may be related to their original environments.
A partial 16S rRNA gene sequence (1420 bp) of strain SB22T showed the highest degree of similarity to the three reference strains, i.e. S. aerolata 5416T-32T (97.3 % similarity), S. parooensis ACM 2042T (95.8 %) and S. xinjiangensis 10-1-101T (92.9 %). The phylogenetic tree reconstructed using the neighbour-joining algorithm revealed that strain SB22T was closely related to the three members of the genus Skermanella and grouped in the same cluster with S. aerolata 5416T-32T, S. parooensis ACM 2042T and S. xinjiangensis 10-1-101T (Fig. 1). The maximum-parsimony tree and the maximum-likelihood tree (Figs S2 and S3) supported the phylogenetic position obtained with the neighbour-joining tree. The DNA–DNA hybridization value between strain SB22T and S. aerolata KACC 11604T was 43.3 % (±0.7; n=3).

The DNA G + C content of the strain SB22T was 69.6 mol%. The major respiratory quinone of strain SB22T and the three reference strains was Q-10. A small amount of Q-8 was also detected (Fig. S4). The major cellular fatty acids (>5 %) of strain SB22T were C₁₈:₁ω7c (65.5 %), summed feature 2 (C₁₄:₀ 3-OH and/or iso-C₁₆:₁ 1; 10.8 %) and C₁₆:₀ (9.9 %) on R2A medium (details are shown in Table 2), which were similar to those found in recognized species of the genus Skermanella. Polar lipids found in strain SB22T and the three reference strains were diphosphatidylglycerol, phosphatidylycholine, phosphatidylethanolamine and phosphatidylglycerol, an unknown aminolipid and small amounts of two unknown phospholipids (Fig. S5). The chemotaxonomic characteristics of strain SB22T were very similar to those of other species of the genus Skermanella. There were also some identical biochemical and physiological characteristics among the species of this genus (details are given in the emended description of the genus). In particular, they were unable to perform acetylene reduction in N₂-free malate medium, which indicated that they were unable to fix nitrogen. This clearly distinguished them from members of the genus Azospirillum, which are able to fix nitrogen.

On the basis of the close relationship with reference strains and the distinctive phenotypic, genotypic and phylogenetic differences, it is concluded that strain SB22T represents a novel species, for which the name Skermanella stibiiresistens sp. nov., is proposed.

**Emended description of the genus Skermanella**

This emended description is based on that by Sly & Stackebrandt (1999) and Weon et al. (2007) with the following changes. All members of the genus are positive for catalase, oxidase, alkaline phosphatase, acid phosphatase, esterase (C4), naphthol-AS-BI-phosphohydrolase and leucine arylamidase, but negative for β-galactosidase, β-galactosidase, β-mannosidase, β-fucosidase, N-acetyl-β-glucosaminidase, α-chymotrypsin and trypsin. Glucose fermentation is variable (corrected from a previous genus description; Weon et al., 2007). Strains of this genus have a high DNA G + C content (65.0–69.6 mol%) and the major respiratory quinone is Q-10. The major polar lipids are diphosphatidylglycerol, phosphatidylycholine, phosphatidylethanolamine, phosphatidylglycerol and an unknown aminolipid. The type species is Skermanella parooensis.

**Description of Skermanella stibiiresistens sp. nov.**


Cells are Gram-negative, aerobic, motile and rod-shaped. Colonies grown on R2A agar are convex, circular, smooth and light-pink-coloured. Grows well on LB agar, R2A agar,
trypticase soy agar and MacConkey agar. Temperature range for growth is 4–37 °C (optimum at 28 °C). Growth occurs with NaCl concentrations in the range 0–4% and pH 5–9 (optimum, pH 7). Oxidase- and catalase-positive. Hydrolyses tyrosine and Tween 40, but not gelatin, starch, casein, DNA, carboxymethyl-cellulose or Tween 80. H₂S is not produced. Negative for methyl red and Voges–Proskauer tests. Positive reaction for β-galactosidase, but negative reactions for nitrate reduction, indole production, urease, arginine dihydrolase, aesculin hydrolysis and glucose fermentation (API 20 NE test strips). The following substrates are assimilated as sole carbon sources: D-glucose, D-ribose, rhamnose, L-proline, salicin, inositol, DL-lactate, L-alanine, malic acid, potassium 2-ketogluconate and 3-hydroxybutyric acid. The following substrates are not utilized: sucrose, maltose, L-arabinose, mannose, melibiose, L-fucose, glycogen, mannitol, D-sorbitol, N-acetylglucosamine, capric acid, valeric acid, itaconic acid, phenylacetic acid, 3-hydroxybenzoic acid, L-serine, histidine, trisodium citrate, propionate, 4-hydroxybenzoate, suberate, malonate, acetate, lactate and potassium 5-ketogluconate (API 20 NE and API ID 32 GN). Positive for α-glucosidase (weak), β-glucosidase (weak), alkaline phosphatase, acid phosphatase, esterase (C4), leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for α-galactosidase, β-galactosidase, β-glucuronidase, α-chymotrypsin, α-mannosidase, α-fucosidase, esterase lipase (C8), lipase (C14), cystine arylamidase, N-acetyl-β-glucosaminidase and trypsin (API ZYM). Acid is produced from D-glucose, D-ribose, L-rhamnose, D-galactose, mannose, D-xylene and turanose, but not from sucrose, lactose, maltose, D-arabinose, D-fructose, cellobiose, raffinose, trehalose, L-sorbos, melezitose, inositol, D-sorbitol, L-sorbose or D-mannitol. Sensitive to (m)G. Luo and others

**Table 2.** Cellular fatty acid contents (%) of strain SB22ᵀ and type strains of the three species in the genus Skermanella

<table>
<thead>
<tr>
<th>Fatty acid</th>
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<td>3.2</td>
<td>1.3</td>
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<td>C₁₆:₀</td>
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<td>12.3</td>
<td>12.7</td>
<td>6.5</td>
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<tr>
<td>C₁₆:₁ 3-OH</td>
<td>3.1</td>
<td>3.1</td>
<td>1.1</td>
<td>2.2</td>
</tr>
<tr>
<td>C₁₆:₁ 011c</td>
<td>2.9</td>
<td>3.9</td>
<td>5.9</td>
<td>4.1</td>
</tr>
<tr>
<td>C₁₇:₁ 06c</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.9</td>
</tr>
<tr>
<td>C₁₈:₀</td>
<td>0.7</td>
<td>–</td>
<td>–</td>
<td>2.3</td>
</tr>
<tr>
<td>C₁₈:₁ 07c</td>
<td>63.5</td>
<td>58.8</td>
<td>59.9</td>
<td>65.4</td>
</tr>
<tr>
<td>C₁₈:₁ 2-OH</td>
<td>2.5</td>
<td>–</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>C₁₈:₁ 09c</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
<td>1.0</td>
</tr>
<tr>
<td>Summed features*</td>
<td>10.8</td>
<td>9.6</td>
<td>9.8</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>4.2</td>
<td>9.0</td>
<td>5.5</td>
<td>1.5</td>
</tr>
</tbody>
</table>

*Summed feature 2 contains C₁₄:₀ 3-OH and/or iso-C₁₆:₁ I; summed feature 3 contains iso-C₁₅:₀ 2-OH and/or C₁₆:₁ 07c.

Hydrolyses tyrosine and Tween 40, but not gelatin, starch, casein, DNA, carboxymethyl-cellulose or Tween 80. H₂S is not produced. Negative for methyl red and Voges–Proskauer tests. Positive reaction for β-galactosidase, but negative reactions for nitrate reduction, indole production, urease, arginine dihydrolase, aesculin hydrolysis and glucose fermentation (API 20 NE test strips). The following substrates are assimilated as sole carbon sources: D-glucose, D-ribose, rhamnose, L-proline, salicin, inositol, DL-lactate, L-alanine, malic acid, potassium 2-ketogluconate and 3-hydroxybutyric acid. The following substrates are not utilized: sucrose, maltose, L-arabinose, mannose, melibiose, L-fucose, glycogen, mannitol, D-sorbitol, N-acetylglucosamine, capric acid, valeric acid, itaconic acid, phenylacetic acid, 3-hydroxybenzoic acid, L-serine, histidine, trisodium citrate, propionate, 4-hydroxybenzoate, suberate, malonate, acetate, lactate and potassium 5-ketogluconate (API 20 NE and API ID 32 GN). Positive for α-glucosidase (weak), β-glucosidase (weak), alkaline phosphatase, acid phosphatase, esterase (C4), leucine arylamidase, valine arylamidase and naphthol-AS-BI-phosphohydrolase, but negative for α-galactosidase, β-galactosidase, β-glucuronidase, α-chymotrypsin, α-mannosidase, α-fucosidase, esterase lipase (C8), lipase (C14), cystine arylamidase, N-acetyl-β-glucosaminidase and trypsin (API ZYM). Acid is produced from D-glucose, D-ribose, L-rhamnose, D-galactose, mannose, D-xylene and turanose, but not from sucrose, lactose, maltose, D-arabinose, D-fructose, cellobiose, raffinose, trehalose, L-sorbose, melezitose, inositol, D-sorbitol, L-sorbose or D-mannitol. Sensitive to (m)G. Luo and others

**Fig. 1.** A neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain SB22ᵀ among closely related neighbours. Numbers indicate percentages of occurrence of the branching order in 1000 bootstrapped trees. Bar, 1 substitution per 100 nt.
polymyxin B (25), minocin (30), kalafungin (30), rifampicin (5), neomycin (30), carbenicillin (100), amoxicillin (10), penicillin (10), chloramphenicol (30), nalidixic acid (30), ofloxacin (5), norfloxacin (10), cephalosporin V (30), cephalosporin IV (30), cefalotin (30), erythromycin (15), tobramycin (10), streptomycin (10), cefoxitin (30) and tetracycline (30). Q-10 is the major respiratory quinone. The major cellular fatty acids (>5%) are C18:1ω7c, summed feature 2 (C14:0 3-OH and/or iso-C16:1 I) and C16:0. The major polar lipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and an unknown aminolipid. Highly antimonity-resistant with the ability to grow in 4 mM Sb(III) in R2A broth.

The type strain, SB23T (=CGMCC 1.10751T=KCTC 23364T), was isolated from soil of Jixi coal mine, Jixi City, Heilongjiang Province, north-east China. The DNA G+C content of the type strain is 69.6 mol%.

Acknowledgements

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References


