Microvirga guangxiensis sp. nov., a novel alphaproteobacterium from soil, and emended description of the genus Microvirga

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A Gram-negative-staining bacterium, designated strain 25BT, was isolated from a soil sample from a rice field in Guangxi Province, China, and its taxonomic position was investigated by using a polyphasic approach. Cells were rod-shaped, non-sporulating, non-motile and strictly aerobic. Strain 25BT grew optimally at 37 °C and pH 7.0. The predominant fatty acids of this soil isolate were C₁₈:₁ω7c, C₁₈:₀ cyclo ω8c and C₁₈:₀. Phylogenetic analysis based on the almost-complete 16S rRNA gene sequence showed that strain 25BT formed a monophyletic clade with the type strain of Microvirga subterranea; the two organisms shared 97.2% 16S rRNA gene sequence similarity. However, the two strains shared low DNA–DNA relatedness. Strain 25BT also was readily distinguishable from Microvirga subterranea DSM 14364T by various phenotypic characteristics. The combination of genotypic and phenotypic data suggests that the isolate represents a novel species of the genus Microvirga, for which the name Microvirga guangxiensis sp. nov. is proposed. The type strain is 25BT (=CGMCC 1.7666T =JCM 15710T).

The genus Microvirga was established by Kanso & Patel (2003) to accommodate strictly aerobic, Gram-negative, non-sporulating, motile and rod-shaped bacteria from free-flowing geothermal waters of a bore tapping the Great Artesian Basin of Australia, which grew optimally at 41 °C and pH 7.0 and had an absolute requirement for yeast extract. The genus currently encompasses a single species with a validly published name, Microvirga subterranea, and is assigned to the class Alphaproteobacteria, phylum Proteobacteria (Garrity & Holt, 2001; Garrity et al., 2005a), almost equidistant from Methylobacterium species (Patt et al., 1976; Urakami et al., 1993), Chelatococcus asaccharovorans (Auling et al., 1993; Egli et al., 1988) and Bosea thiooxidans (Das et al., 1996), its nearest phylogenetic relatives, with a mean similarity value of 93%.

A sampling campaign during an ecological survey of soil bacteria in China led to the isolation of a pink-pigmented bacterium, designated strain 25BT. The soil sample (10 cm in depth, pH 6.2) was collected from a rice field in Guangxi Province. A glucose-yeast extract-malt extract agar plate (comprising, 1⁻¹: 4 g D-glucose, 4 g yeast extract, 10 g malt extract, 15 g agar; pH 7.3) was inoculated with a suspension of the soil and incubated at 37 °C for 7 days. The isolate that was obtained, strain 25BT, was maintained on LB agar (1⁻¹: 10 g tryptone, 5 g yeast extract, 10 g NaCl, 15 g agar; pH 7.0) at 4 °C and as glycerol suspensions (20%, v/v) at −20 °C. Biomass for the chemotaxonomic and molecular systematic studies was prepared as described previously (Zhang et al., 2002) with the modification that the strain was grown in shake flasks of LB broth.

Micromorphological and colonial properties of strain 25BT were examined on LB agar, Rourf’s agar (Mulder & Deinema, 1992) and GYM agar after incubation at 37 °C for 3–7 days. Phase-contrast and electron microscopy of cells were performed as described by Andrews & Patel (1996). The presence of flagella and spores was assessed by the method of Das et al. (1996) and Kanso & Patel (2003). The presence of poly-β-hydroxybutyrate (PHB) was demonstrated by staining lightly heat-fixed cells with Sudan Black B [0.3% (w/v) in 70% (v/v) ethanol] for 10 min followed by decolourizing with xylene for 10 s, drying and counterstaining with safranin [0.5% (w/v) in water] for 1–2 min.

Strain 25BT was examined for a range of phenotypic properties. Tests for acid production from carbohydrates and utilization of sole carbon sources were carried out according to procedures described elsewhere (Das et al., 1996; Kanso & Patel, 2003; Zhang et al., 2008). Catalase
activity was determined on freshly grown colonies using 3 % (v/v) hydrogen peroxide solution. Determination of starch and casein hydrolysis and oxidative or fermentative catabolism followed the methods described by Snibert & Krieg (1994) and La Scola et al. (2003). Growth with various pH, temperature and sodium chloride regimes was determined on LB agar plates incubated for up to 3 days. Anaerobic growth was tested by using TYEG medium (Wynert et al., 1996). Resistance to lysozyme was determined by using the method of Gordon et al. (1974). Resistance to antibiotics was examined by placing impregnated filter-paper discs (Goodfellow & Orchard, 1974) on LB agar; the results were recorded following incubation at 37 °C for up to 3 days. Microvirga subterranea DSM 14364T was used as the control.

Genomic DNA preparation, PCR-mediated amplification of the 16S rRNA gene sequence and purification of the PCR product from strain 25B1 were carried out after Rainey et al. (1996). The PCR product was sequenced directly using the method of Lu et al. (2001). Sequence gel electrophoresis was performed and the nucleotide sequences were obtained automatically by using an Applied Biosystems DNA Sequencer (model 377) with the software provided by the manufacturer. The resultant 16S rRNA gene sequence was aligned manually against corresponding sequences of representatives of genera classified in the order Rhizobiales (Kuykendall, 2005) that had been retrieved from the GenBank/EMBL/DDBJ databases using CLUSTAL X 1.8 software (Thompson et al., 1997). Phylogenetic trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1981) and neighbour-joining (Saitou & Nei, 1987) algorithms. Evolutionary distance matrices were generated after Kimura (1980). The resultant unrooted topologies were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining dataset, based on 1000 resamplings, using the SEQBOOT and CONSENSE options. The PHYLIP package (Felsenstein, 1993) was used for all phylogenetic analyses.

Respiratory lipoquinones were extracted and purified after Das et al. (1996) and determined by using reversed-phase HPLC (Wu et al., 1989). The fatty acids were extracted, purified, methylated and quantified by GC using the standard Microbial Identification System (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The G+C content of the DNA was determined using the thermal denaturation method (Marmur & Doty, 1962) with Escherichia coli AS 1.365 as the standard. DNA–DNA relatedness between strain 25B1 and Microvirga subterranea DSM 14364T was determined using a thermal denaturation procedure (De Ley et al., 1970; Huß et al., 1983) with a UV-1206 spectrophotometer (Shimadzu) fitted with a TB-85 thermobath and standard software (Jahnke, 1992).

An almost-complete 16S rRNA gene sequence (1397 nt) was obtained for strain 25B1. The phylogenetic analysis showed that the sequence contained all of the signature nucleotides that are expected for members of the order Rhizobiales and, more specifically, those that are characteristic of members of the family Methylobacteriaceae (Garrity et al., 2005b) and the genus Microvirga (Kanzo & Patel, 2003). The high 16S rRNA gene sequence similarity between strain 25B1 and Microvirga subterranea DSM 14364T (97.2 %) provides further support for the isolate’s assignment to the genus Microvirga.

The classification of strain 25B1 in the genus Microvirga was supported by both morphological and phenotypic characteristics. The strain was a strictly aerobic, Gram-negative, non-motile, non-sporulating, catalase-positive bacterium. Colonies (0.5–1.5 mm) were light pink, circular and convex with entire smooth edges and shiny surfaces, thin in texture and easily emulsifiable. The rod-shaped cells (0.6–0.8 x 1.3–2.1 μm) with rounded ends occurred singly and as pairs, tetrads and packets. Large intracellular granules in cells from ageing cultures were observed by phase-contrast microscopy and staining revealed darkly stained PHB granules. Spores were not observed and the notion that strain 25B1 was a non-spore-former was supported by its sensitivity to heat (70 °C for 10 min). The organism did not grow anaerobically in TYEG medium. The doubling time of strain 25B1 under optimal growth conditions was 230 min. Ubiquinone 10 was the major ubiquinone. The non-hydroxylated fatty acid profile of strain 25B1 consisted of straight chain and cyclo saturated, unsaturated and 11-methyl-branched components, mainly containing C18:1ω7c (41.8 %), C19:0 cyclo ω8c (32.1 %), C16:0 (9.0 %), C18:0 (2.8 %), C18:1ω9Tc 11-methyl (1.8 %), C17:0 (1.6 %), C17:0 cyclo (1.4 %), C20:2ω9c (1.2 %), C18:0 3-OH (1.1 %). The fatty acid pattern was similar to that of Microvirga subterranea DSM 14364T but there were notable differences in the levels of C18:1ω9Tc 11-methyl and C17:0 (see Supplementary Table S1, available in IJSEM Online). In addition, the DNA of strain 25B1 was rich in guanine and cytosine (G+C content 64.3 mol%).

The position of strain 25B1 in the unrooted 16S rRNA gene sequence tree, based on three algorithms, is shown in Fig. 1. Strain 25B1 was most closely related to and formed a monophyletic clade with Microvirga subterranea DSM 14364T, and bootstrap analysis gives a high degree of confidence to this relationship (100 %). The 16S rRNA gene similarity value between strain 25B1 and Microvirga subterranea DSM 14364T was 97.2 % (35 differences over 1397 nucleotide positions). Strain 25B1 was also closely related to B. thiooxidans BI 42T (93.5 % similarity; 87 differences), Methylobacterium suomiense F20T (93.3 %; 88 differences) and C. asaccharovorans TE2T (93.1 %; 90 differences). The mean DNA–DNA relatedness value between the novel isolate and the type strain of Microvirga subterranea was 21.7 %, a value well below the 70 % cut-off point recommended by Wayne et al. (1987) for the delineation of genomic species. A range of phenotypic properties separated strain 25B1 from Microvirga subterranea DSM 14364T (Table 1), in particular with its ability to utilize arabitol, (+)-D-glucose, (+)-...
D-mannitol, (+)-melezitose, (+)-D-sorbitol and malonate as carbon sources as well as its inability to hydrolyse gelatin. *Microvirga subterranea* DSM 14364T had a restricted substrate range for growth in comparison with strain 25BT and, unlike *Microvirga subterranea* DSM 14364T, strain 25BT did not have an absolute requirement for yeast extract. Salt tolerance was another characteristic feature for phenotypic differentiation: strain 25BT grew in the presence of 2% (w/v) NaCl and *Microvirga subterranea* DSM 14364T tolerated 1% NaCl and did not grow in the presence of 2% NaCl.

The genotypic and phenotypic data show that strain 25BT merits recognition as a representative of a novel species of the genus *Microvirga*. It is, therefore, proposed that the isolate be classified as *Microvirga guangxiensis* sp. nov.

**Emended description of the genus Microvirga Kanso and Patel 2003**

Strictly aerobic, small, rod-shaped cells that stain Gram-negative and produce light-pink colonies. The optimum temperature for growth is 37–41 °C, with a temperature range for growth between 16 and 45 °C. Grows with or without glucose but uses xylose. Reduces nitrate to nitrite. Cells are susceptible to antibiotics that inhibit members of domain *Bacteria*. Bacteriochlorophyll is absent but carotenoids are present. PHB is produced. Thiosulfate does not stimulate growth. The G+C content of the DNA is 63–65 mol%. 16S rRNA gene sequence analysis indicates that the genus is a member of the family *Methylobacteriaceae*, class *Alphaproteobacteria*, phylum *Proteobacteria*, placed almost equidistantly between *C. asacharivorans* and *B. thiooxidans*, members of the families *Beijerinckiaceae* and *Bradyrhizobiaceae*, respectively, which are its nearest phylogenetic relatives. The type species is *Microvirga subterranea*.

**Description of Microvirga guangxiensis sp. nov.**

*Microvirga guangxiensis* (gu.ang.xi.en’sis. N.L. fem. adj. guangxiensis referring to Guangxi Province, China, the source of the soil from which the species was first isolated).

The species description is based on a single strain. In addition to the properties described for the genus, the following properties apply. Cells are non-motile rods, 0.6–0.8 × 1.3–2.1 μm, occurring singly, and as pairs, tetrads and packets. Spores are not formed. Cells from old cultures are pleomorphic and contain large intracellular granules (PHB). Colonies are light pink, convex, smooth, mucoid and circular with entire margins, 0.5–1.5 mm in diameter after 3 days at 37 °C on LB agar. Grows well on Rourf’s agar and GYM agar. Diffusible pigments are not formed. Catalase-, lipase- and oxidase-positive. Negative for arginine dihydrolase and lysine decarboxylase. Grows at 16–42 °C (optimum, 37 °C) and pH 5.0–9.5 (optimum, pH 7.0). Urea is hydrolysed, but not aesculin, casein, gelatin or starch. Nitrate is reduced. Indole, methyl red and Voges–Proskauer reactions are negative. Hydrogen sulfide is not produced. Very weak acid production is detected from (+)-D-arabinose but not from (+)-D-glucose, (+)-maltose, (+)-D-mannitol, peptone or (+)-D-xylose. Arabitol, (+)-D-glucose, *myo*-inositol (weak), (+)-maltose (weak), (+)-D-mannitol, (+)-melezitose, (+)-melibiose (weak), peptone, (+)-D-sorbitol, (+)-D-xylose,
Table 1. Characteristics that distinguish strain 25BT (Microvirga guangxiensis sp. nov.) from the type strain of Microvirga subterranea

Data are from this study and Kanso & Patel (2003). Both strains are rod-shaped and positive for utilization of D-xylene and acetate as sole carbon sources. Both strains are negative for hydrolysis of casein and starch, stimulation of growth by thiosulfate and utilization of \(N,N\)-dimethylformamide, ethanol, D-fructose, glycerol and citrate as sole carbon sources. +, Positive; −, negative; w, weak.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain 25BT (^T)</th>
<th>Microvirga subterrane DSM 14364 (^T)</th>
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<tbody>
<tr>
<td>Isolation source</td>
<td>Soil</td>
<td>Water</td>
</tr>
<tr>
<td>Cell dimensions ((\mu)m)</td>
<td>0.6–0.8 × 1.3–2.1</td>
<td>1.0 × 1.5–4.0</td>
</tr>
<tr>
<td>Conditions for growth</td>
<td></td>
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<tr>
<td>Temperature range (°C)</td>
<td>16–42</td>
<td>25–45</td>
</tr>
<tr>
<td>Optimum temperature (°C)</td>
<td>37</td>
<td>41</td>
</tr>
<tr>
<td>NaCl tolerance (% w/v)</td>
<td>&lt;2</td>
<td>&lt;1</td>
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<tr>
<td>Enzyme activities</td>
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<td></td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>+</td>
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<td>Urease</td>
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<tr>
<td>Hydrolysis of gelatin</td>
<td>−</td>
<td>+</td>
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<td>Carbon source utilization</td>
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<tr>
<td>Arabinol</td>
<td>+</td>
<td>−</td>
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<tr>
<td>D-Glucose</td>
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<tr>
<td>(\alpha)-L-Rhamnose</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Acid production from D-glucose</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>64.3 ± 0.5</td>
<td>63.5 ± 0.5</td>
</tr>
</tbody>
</table>

Acetate, fumarate, gluconate (weak), malate and malonate are used as sole carbon and energy sources, but not (+)-D-arabinose, (+)-D-cellulose, \(N,N\)-dimethylformamide, ethanol, (+)-D-fructose, (+)-D-galactose, methyl \(\alpha\)-D-glucopyranoside, glycerol, (+)-lactose, \(\alpha\)-L-rhamnose, (+)-trehalose, benzoate, citrate, oxalate or tartrate. Growth occurs in the presence of sodium chloride at 2%, but not at 2.5% (w/v). Resistance is shown to (\(\mu\)g per disc unless otherwise stated) aztreonam (30), erythromycin (15) and kanamycin sulphate (30). Susceptible to amikacin (30), amoxicillin plus clavulanic acid (10), ampicillin (10), cefotaxime (30), ciprofloxacin (5), clindamycin hydrochloride (2), mezlocillin (75), ofloxacin (5), penicillin G (10 IU), rifampicin (5), streptomycin sulphate (10), tetracycline hydrochloride (30), tobramycin sulphate (10) and lysozyme (0.005 %, w/v). Major fatty acids are \(C_{18:1\ omega7c}\), \(C_{19:0\ cyclo\ \varepsilon8c}\) and \(C_{16:0\ cyclo\ \varepsilon4c}\). Ubiquinone 10 is the major ubiquinone. The DNA G+C content of the type strain is 64.3 mol%.

The type strain, 25BT \(^T\) (=CGMCC 1.7666 \(^T\)=JCM 15710 \(^T\)), was isolated from a rice-field soil sample collected in Guangxi Province, China.

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References


