

Luteimonas terricola sp. nov., a psychrophilic bacterium isolated from soil

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Strain BZ92rT was isolated from hydrocarbon-contaminated soil. Cells were Gram-negative, aerobic, rod-shaped and cold-adapted (growth at 1–25 °C). The major fatty acids were iso-C15 : 0 (25.6 %), iso-C17 : 09c (24.9 %), iso-C11 : 0 (18.4 %) and iso-C11 : 0 3-OH (16.2 %). The predominant ubiquinone was ubiquinone-8. The genomic DNA G+C content was 72.0 mol%. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain BZ92rT was a member of the genus Luteimonas (94.5–95.2 % 16S rRNA gene sequence similarity). On the basis of phenotypic, chemotaxonomic and phylogenetic distinctiveness, strain BZ92rT was considered to represent a novel species of the genus Luteimonas. The name Luteimonas terricola sp. nov. is proposed, with BZ92rT (=DSM 22344T=CGMCC 1.8985T) as the type strain.

The genus Luteimonas was first proposed by Finkmann et al. (2000) to accommodate bacteria that are aerobic, Gram-negative rods that contain ubiquinone-8 as the major ubiquinone and iso-C15 : 0 as the major fatty acid. Currently, the genus Luteimonas includes four species: Luteimonas mephitis (Finkmann et al., 2000), L. composti (Young et al., 2007), L. aquatica (Chou et al., 2008) and L. marina (Baik et al., 2008). The type strains of these species were respectively isolated from ammonia-supplied biofilters in Germany, compost generated from food waste in Taiwan, fresh water from southern Taiwan and seawater from Korea. They are all mesophilic. In this study, we report on the characterization of a novel bacterium, strain BZ92rT, of the genus Luteimonas. The strain was isolated from soil from an industrial site contaminated with hydrocarbons and is the first psychrophilic representative of the genus Luteimonas, i.e. it is well adapted to low temperatures (Margesin et al., 2008) and able to grow at temperatures as low as 1 °C. We use the term ‘psychrophile’ as a general term that describes a micro-organism that grows in a cold environment. The use of growth rates to define the optimum growth temperature, as described by Morita (1975), has been shown to be ambiguous and inappropriate (Feller & Gerday, 2003; Cavicchioli, 2006).

Strain BZ92rT was isolated from soil from an industrial site contaminated with large amounts of heavy oil, located in Bozen, South Tyrol, Italy. The soil was collected under sterile conditions in spring 2008. A soil sample (10 g) was shaken with 90 ml sterile 1 % sodium pyrophosphate (pH 8.5) for 20 min at 150 r.p.m. Appropriate dilutions, prepared with sterile 0.9 % NaCl, were plated (0.1 ml) on a low-strength medium, R2A agar (0.05 % yeast extract, 0.05 % peptone, 0.05 % Casamino acids, 0.05 % glucose, 0.05 % starch, 0.03 % sodium pyruvate, 0.03 % K2HPO4, 0.005 % MgSO4, 1.5 % agar; pH 7), and incubated at 20 °C. One of the pure cultures subsequently obtained was yellow pigmented and was designated BZ92rT. Strain BZ92rT was routinely cultivated in R2A medium at 20 °C. For use as a reference strain, L. mephitis DSM 12574T was routinely grown on nutrient agar (0.5 % peptone, 0.3 % meat extract, 1.5 % agar; pH 7) at 30 °C.

DNA was extracted and purified as described by Sambrook et al. (1989). The gene encoding 16S rRNA was amplified by PCR with two universal primers (Zhang et al., 2006). PCR products were cloned into pGEM-T vectors using the pGEM-T easy vector system (Promega), according to the manufacturer’s instructions. Sequencing reactions were carried out using ABI Big Dye 3.1 sequencing kit and an automated DNA sequencer (ABI 3730; Applied BioSystems). The nearly complete (1537 nt) 16S rRNA gene sequence of strain BZ92rT was submitted to GenBank and EMBL to search for similar sequences using the BLAST algorithm. A phylogenetic tree was constructed using Kimura’s two-parameter and pairwise-deletion model analysis implemented in the program MEGA version 3.0 (Kumar et al., 2004). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates. The phylogenetic analysis (Fig. 1) showed that strain BZ92rT was grouped with members of the genus Luteimonas and formed a distinct cluster with L. mephitis B1953/27,1T (16S rRNA gene sequence similarity 94.9 %). Similar tree topologies were
found in the tree generated with the maximum-parsimony algorithm (data not shown). Lower similarity values (<97.0%) were found with the type strains of other Luteimonas species. It has been suggested that levels of DNA–DNA relatedness less than 70% are observed between strains with less than 97% 16S rRNA gene sequence similarity (Stackebrandt & Goebel, 1994). Therefore, strain BZ92rT is genotypically distinct from other recognized species of the genus Luteimonas.

The cell morphology was examined by phase-contrast microscopy (∼1000). The Gram reaction was determined by Gram staining and confirmed by the KOH lysis test. API tests (API 20E, API 20NE, API ZYM; bioMérieux) were performed at 20 °C to determine physiological and biochemical characteristics, according to the manufacturer’s instructions, and the API M system was used to evaluate cell motility. Sensitivity to antibiotics was determined on R2A agar supplemented with various antibiotics at 20 °C. The morphological, physiological and biochemical characteristics of strain BZ92rT are given in the species description and Table 1.

Respiratory quinones were extracted and purified according to Collins (1985) and were analysed by HPLC (Wu et al., 1989), using ubiquinone-8 from L. mephitis DSM 12574T as a reference. Strain BZ92rT contained ubiquinone-8 as the major quinone. For fatty acid methyl ester analysis, cell biomass of strain BZ92rT was harvested from tryptic soy agar (TSA) plates after incubation at 20 °C for 2 days. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the Microbial Identification system (Sasser, 1990). The major fatty acids were iso-C15:0 (25.6%), iso-C17:1ω9c (24.9%), iso-C11:0 (18.4%) and iso-C11:0 3-OH (16.2%). The fatty acid profile of strain BZ92rT differs from those of other Luteimonas species by having larger proportions of iso-C11:0 and iso-C11:0 3-OH (Finkmann et al., 2000; Young et al., 2007; Baik et al., 2008; Chou et al., 2008). The fatty acid profiles of strain BZ92rT and the type strains of Luteimonas species are shown in Table 2.

The G+C content of the DNA was tested by the thermal denaturation method (Sly et al., 1986) with DNA from Escherichia coli K-12 as the reference, using a model Lambda 35 UV/Vis spectrometer equipped with a temperature program controller (PerkinElmer). The DNA G+C content of strain BZ92rT was 72.0 mol%.

Strain BZ92rT differs from the type strains of the other species of the genus Luteimonas mainly by its psychrophilic growth characteristics: i.e. the strain is able to grow at low temperatures (down to 1 °C), while no growth occurs at 30 °C, which has not yet been described for other Luteimonas species. According to its physiological and phylogenetic properties, the strain is closely related to L. mephitis, the type species of the genus. Based on the phenotypic, phylogenetic and genomic evidence, strain BZ92rT was identified as a representative of a novel species of Luteimonas, for which the name Luteimonas terricola sp. nov. is proposed.

Description of Luteimonas terricola sp. nov.

Luteimonas terricola (ter.ri.co.la. L. n. terra earth, soil; L. suff. -cola inhabitant, dweller; N.L. n. terricola a dweller
upon earth, soil-dweller, referring to the isolation of the type strain from soil).

Cells are aerobic, Gram-negative, non-spore-forming, non-motile and rod-shaped (0.6–0.8 × 1.8–2.2 μm in R2A medium). Colonies on R2A agar are light yellow, round, convex, opaque and smooth with entire margins, approx. 1–1.5 mm in diameter. Growth occurs in R2A medium at 1–25 °C, with the highest cell yields at 10–15 °C and the fastest growth at 25 °C; growth on R2A agar at 1 °C is very weak. Growth on R2A agar occurs at pH 7–8 and with 0–3 % (w/v) NaCl. Produces catalase and cytochrome oxidase but not indole, H2S or urease. Reduces nitrate. Does not ferment glucose or other carbon sources in the API 50CH B/E system. Resistant to ampicillin and penicillin G, but sensitive to chloramphenicol, kanamycin, rifampicin, streptomycin and tetracycline (all at 30 μg ml⁻¹). Ubiquinone-8 is the major ubiquinone. The predominant cellular fatty acids are iso-C₁₅:₀, iso-C₁₇:₀, iso-C₁₆:₁ and iso-C₁₄:₀. The DNA G+C content of the type strain is 72.0 mol%.

The type strain is BZ92rᵀ (=DSM 22344ᵀ =CGMCC 1.8983ᵀ), isolated from hydrocarbon-contaminated soil in Bozen, South Tyrol, Italy.

**Acknowledgements**

This research work was supported by a grant from the Autonome Provinz Bozen, Südtirol. We thank P. Thurnbichler for technical assistance.
References


