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

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Strain BZ92r^T 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-C_{15:0} (25.6 %), iso-C_{17:1ω9c} (24.9 %), iso-C_{11:0} (18.4 %) and iso-C_{11:0} 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 BZ92r^T 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 BZ92r^T was considered to represent a novel species of the genus *Luteimonas*. The name *Luteimonas terricola* sp. nov. is proposed, with BZ92r^T (=DSM 22344^T =CGMCC 1.8985^T) 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-C_{15:0} 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 BZ92r^T, 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 BZ92r^T 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 BZ92r^T. Strain BZ92r^T was routinely cultivated in R2A medium at 20 °C. For use as a reference strain, *L. mephitis* DSM 12574^T 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 BZ92r^T 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 BZ92r^T was grouped with members of the genus *Luteimonas* and formed a distinct cluster with *L. mephitis* B1953/27.1^T (16S rRNA gene sequence similarity 94.9 %). Similar tree topologies were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain BZ92r^T is FJ948107.

Fig. 1. Neighbour-joining tree, based on 16S rRNA gene sequence data, showing the phylogenetic position of strain BZ92r^T, recognized members of the genus *Luteimonas* and representatives of some related taxa. Percentages at nodes are levels of bootstrap support based on 1000 replications. Bar, 1 % sequence divergence.

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 BZ92r^T is genotypically distinct from other recognized species of the genus *Luteimonas*.

The cell morphology was examined by phase-contrast microscopy ($\times 1000$). 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 BZ92r^T 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 12574^T as a reference. Strain BZ92r^T contained ubiquinone-8 as the major quinone. For fatty acid methyl ester analysis, cell biomass of strain BZ92r^T 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-C_{15:0} (25.6 %), iso-C_{17:1}ω9c (24.9 %), iso-C_{11:0} (18.4 %) and iso-C_{11:0} 3-OH (16.2 %). The fatty acid profile of strain BZ92r^T differs from those of other *Luteimonas* species by having larger proportions of iso-C_{11:0} and iso-C_{11:0} 3-OH (Finkmann *et al.*, 2000; Young *et al.*, 2007; Baik *et al.*, 2008; Chou *et al.*, 2008). The fatty acid profiles of strain BZ92r^T 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 BZ92r^T was 72.0 mol%.

Strain BZ92r^T 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 BZ92r^T 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

Table 1. Phenotypic characteristics that differentiate strain BZ92r^T from the type strains of *Luteimonas* species

Strains: 1, *Luteimonas terricola* sp. nov. BZ92r^T; 2, *L. aquatica* LMG 24212^T (data from Chou *et al.*, 2008); 3, *L. composti* CC-YY255^T (Young *et al.*, 2007); 4, *L. marina* JCM 12488^T (Baik *et al.*, 2008); 5, *L. mephitis* DSM 12574^T (Finkmann *et al.*, 2000; Chou *et al.*, 2008). All strains were positive for aerobic growth and catalase, oxidase, alkaline and acid phosphatases, esterase (C4), esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase activities. All strains were negative for the Gram reaction, spore formation, motility, glucose fermentation, production of indole and urease, lipase (C14), arginine dihydrolase, lysine dihydrolase, ornithine dihydrolase, α -fucosidase, α -galactosidase and β -glucuronidase activities and adipate, caprate and phenylacetate assimilation. +, Positive; w, weak; –, negative; ND, no data available.

Characteristic	1	2	3	4	5
Temperature range for growth (°C)	1–25	15–37	20–45	20–42	$T_{\max}$ 37
Nitrate reduction	+	–	+	–	–
Hydrolysis of:					
Aesculin	–	ND	+	+	–
Gelatin	–	ND	+	+	+
Enzyme assays (API ZYM)					
α -Chymotrypsin	w	–	+	+	–
N-Acetyl- β -glucosaminidase	–	+	+	+	–
α -Mannosidase	–	–	–	+	–
Cystine arylamidase	–	+	–	–	–
Valine arylamidase	–	+	+	+	+
Assimilation of:					
Arabinose	–	–	+	+	–
Citrate	–	–	–	+	–
Malate	–	+	–	+	–
D-Glucose	–	+	+	+	–
Maltose	–	+	+	+	–
D-Mannose	–	+	–	–	–
Tween 80	–	+	–	w	+
DNA G + C content (mol%)	72.0	70.3	68.1	67.6	67.0

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 ($0.6\text{--}0.8 \times 1.8\text{--}2.2\ \mu\text{m}$ 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 hydrolyse aesculin or gelatin. Positive for alkaline phosphatase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase, weakly positive for esterase (C4), esterase lipase (C8), acid phosphatase, trypsin and α -chymotrypsin, but negative for lipase (C14), cystine arylamidase, arginine

Table 2. Comparison of cellular fatty acid compositions of strain BZ92r^T and the type strains of *Luteimonas* species

Strains: 1, *L. terricola* sp. nov. BZ92r^T; 2, *L. aquatica* LMG 24212^T (data from Chou *et al.*, 2008); 3, *L. composti* CC-YY255^T (Young *et al.*, 2007); 4, *L. marina* JCM 12488^T (Baik *et al.*, 2008); 5, *L. mephitis* DSM 12574^T (Young *et al.*, 2007). Values are percentages of total fatty acids.

Fatty acid	1	2	3	4	5
Straight-chain saturated					
C _{14:0}	0.5	0.2	–	0.7	0.7
C _{16:0}	0.9	2.4	4.7	5.9	1.6
Branched saturated					
iso-C _{10:0}	0.1	–	0.4	–	0.1
iso-C _{11:0}	18.4	4.0	6.5	6.4	5.2
anteiso-C _{11:0}	0.1	–	0.3	0.2	0.2
iso-C _{13:0}	0.4	0.2	–	0.1	0.5
iso-C _{14:0}	0.3	0.3	1.9	0.2	1.5
iso-C _{15:0}	25.6	25.3	25.5	26.2	47.4
anteiso-C _{15:0}	0.7	6.2	2.7	4.5	3.0
iso-C _{16:0}	0.8	4.4	13.8	1.7	5.6
iso-C _{17:0}	3.9	21.5	5.5	14.5	5.5
Unsaturated					
iso-C _{15:1}	3.6	–	1.9	–	3.0
iso-C _{17:1ω9c}	24.9	22.3	25.5	26.0	18.7
Hydroxy					
iso-C _{11:0} 3-OH	16.2	6.7	5.5	4.7	5.3
Summed feature 3*	2.6	1.4	5.5	2.2	0.9

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 consisted of C_{16:1 ω 7c} and/or iso-C_{15:0} 2-OH.

dihydrolase, lysine dihydrolase, ornithine dihydrolase, tryptophan deaminase, valine arylamidase, α -fucosidase, α -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, N-acetyl- β -glucosaminidase and α -mannosidase. Does not utilize L-arabinose, D-glucose, maltose, D-mannitol, D-mannose, N-acetylglucosamine, gluconate, citrate, adipic acid, capric acid, malic acid or phenylacetic acid as carbon sources. 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 $\mu\text{g ml}^{-1}$). Ubiquinone-8 is the major ubiquinone. The predominant cellular fatty acids are iso-C_{15:0}, iso-C_{17:1 ω 9c}, iso-C_{11:0} and iso-C_{11:0} 3-OH. The DNA G + C content of the type strain is 72.0 mol%.

The type strain is BZ92r^T (=DSM 22344^T =CGMCC 1.8985^T), isolated from hydrocarbon-contaminated soil in Bozen, South Tyrol, Italy.

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