

Solirubrobacter pauli gen. nov., sp. nov., a mesophilic bacterium within the *Rubrobacteridae* related to common soil clones

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A novel bacterium, strain B33D1^T, isolated from agricultural soil, was characterized taxonomically and phylogenetically. Strain B33D1^T was a Gram-positive, aerobic rod of medium length that formed long chains on a common laboratory medium. However, B33D1^T grew poorly on the surface of agar plates and was sensitive to desiccation. The optimal growth temperature was 30 °C (range 19–38 °C). The organism grew well on a variety of sugars and was capable of utilizing a few amino acids as sole carbon sources. Phylogenetically, the most closely related described species to strain B33D1^T was *Rubrobacter xylanophilus*, which possessed 86% 16S rRNA sequence similarity. However, a number of 16S rRNA gene clones derived from soil samples possessed up to 93% sequence similarity. These results placed strain B33D1^T within the subclass *Rubrobacteridae* of the phylum *Actinobacteria*. The novel genus and species *Solirubrobacter pauli* gen. nov., sp. nov. is proposed, with strain B33D1^T (=ATCC BAA-492^T=DSM 14954^T) as the type strain.

INTRODUCTION

Members of the phylum *Actinobacteria* are widespread in soils throughout the world. In recent years, a number of 16S rRNA gene libraries constructed from terrestrial samples have revealed sequences that were phylogenetically similar to two isolates within a deep branch of the *Actinobacteria*, *Rubrobacter xylanophilus* (Carreto *et al.*, 1996) and *Rubrobacter radiotolerans* [originally *Arthrobacter radiotolerans* (Yoshinaka *et al.*, 1973); later reclassified by Suzuki *et al.* (1988)]. Both of these organisms are notable for their radiation tolerance, rare pigmentation and thermophily. In contrast to the extreme environments that were the sources of the described *Rubrobacter* species, a large number of related 16S rRNA gene sequences have been recovered from moderate, terrestrial environments (Furlong *et al.*, 2002; Holmes *et al.*, 2000; McCaig *et al.*, 1999; Rheims *et al.*, 1996; Ueda *et al.*, 1995), suggesting a wider range of habitat for the group. In this paper, we characterize

an organism that is closely related to these common, uncultured soil bacteria within the *Rubrobacteridae*.

METHODS

Isolation. Strain B33D1^T was isolated from a burrow of the epigeic earthworm *Lumbricus rubellus* in an agricultural soil during a previous study (Furlong *et al.*, 2002), on a plate composed of 50% Difco nutrient broth medium (NB; pH 7.0, ~23 °C). Isolates were allowed to grow for 2 weeks before colonies were picked. A single pink colony was selected from a dilution series and maintained on the same medium. The organism was stored at –70 °C in medium containing 15% glycerol.

Growth conditions. Unless otherwise indicated, the optimization of growth conditions for B33D1^T was carried out in undiluted Difco nutrient broth or NB medium. The temperature range was determined using a temperature gradient incubator (Scientific Industries Inc.). The pH range of B33D1^T was determined by buffering NB with 25 mM MES (pH 5.5, 6.0 and 6.5), HEPES (pH 7.0, 7.5 and 8.0), EPPS (pH 8.5) or CHES (pH 9.0, 9.5, 10.0). The pH of the medium did not change during growth. Growth in various salt concentrations was tested by adding 0–10% NaCl to NB. Growth under anaerobic conditions was tested by sparging NB with N₂ gas for 30 min to remove O₂ and incubating cultures in Balch tubes (Balch *et al.*, 1979). To test growth under microaerophilic conditions, 1% (v/v) air was added to N₂-sparged tubes of NB. The effect of

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Abbreviations: NB, nutrient broth; PLFA, phospholipid fatty acid.

The GenBank/EMBL/DDBJ accession number for the nearly complete 16S rRNA gene sequence of strain B33D1^T is AY039806.

increased CO₂ concentrations on B33D1^T was tested by adding 1–5% (v/v) CO₂ to air in the headspace of NB medium in Balch tubes.

Biochemical properties. The ability of various compounds to serve as sole carbon sources was tested in minimal medium with the carbon source added to a final concentration of 0.2% (w/v). The minimal medium consisted of 1 mM K₂HPO₄, 2 mM NH₄NO₃, 1 mM MgSO₄, 1% (v/v) trace mineral solution (Whitman *et al.*, 1986) and 1.0% (v/v) iron solution (Whitman *et al.*, 1986) adjusted to pH 7.0. In some tests (for example, the carbon utilization of lignin-associated compounds), the high concentration of iron produced a precipitate, and a 10-fold lower concentration was used. Tubes were incubated at 30 °C for 2 weeks before growth results were recorded.

The oxidase reaction of B33D1^T was tested by applying a few drops of BBL Oxidase test reagent (Becton-Dickinson) to cells on a piece of filter paper. Other tests were performed as described by Furlong *et al.* (2002).

Desiccation resistance. Strain B33D1^T and *Deinococcus radiodurans* ATCC 35073 were grown in liquid media (NB for B33D1^T, NB+10% glucose for *D. radiodurans*) at 30 °C until growth was apparent. Wild-type *Escherichia coli* ATCC 9637 was grown in NB. Samples of the cultures (1–3 ml) were centrifuged for 3 min and the pellets were resuspended in 0.4 ml PBS (pH 7.2). Samples (0.1 ml) were then aliquotted into microfuge tubes, the tubes were centrifuged for 3 min and the buffer was discarded. The open microfuge tubes containing the cell pellets were placed upright in a sealed Mason jar containing Drierite absorbent for up to 25 days. Tubes were removed at various intervals and the cell pellets were resuspended and serially diluted in PBS. *D. radiodurans* was spread onto plates of NB+10% glucose. *E. coli* was spread onto NB plates. For enumeration of B33D1^T, 1 ml cell suspension was added to 9 ml molten agar medium (NB+1.5% agar, 55 °C) and poured into a sterile Petri dish. Once the plates had solidified, they were sealed with Parafilm and incubated at 30 °C until colonies became visible.

Fatty acid analysis. A sample of isolate B33D1^T was analysed to determine the phospholipid fatty acid (PLFA) content of the organism. The cell paste was extracted with the single-phase chloroform/methanol buffer system of Bligh & Dyer (1954), as modified by White *et al.* (1979). The total lipid extract was fractionated into neutral lipids, glycolipids and polar lipids by silicic acid column chromatography (Guckert *et al.*, 1985). The polar lipids were transesterified to the fatty acid methyl esters by a mild alkaline methanolysis (Guckert *et al.*, 1985). The fatty acid methyl esters were then analysed by capillary GC with flame-ionization detection on a Hewlett Packard 5890 Series 2 chromatograph with a 50 m non-polar column (0.2 mm i.d., 0.11 µm film thickness). The injector and detector were respectively maintained at 270 and 290 °C. The column temperature was programmed at 60 °C for 2 min, ramped at 10 °C min⁻¹ to 150 °C and finally ramped to 312 °C at 3 °C min⁻¹. Preliminary peak identification was by comparison of retention times with known standards. Detailed identification of peaks was by GC/MS of selected samples using a Hewlett Packard 5890 series 2 GC interfaced to a Hewlett Packard 5971 mass-selective detector using the same column and temperature program as described previously. Mass spectra were determined by electron impact at 70 eV. Methyl nonadecanoate (C19:0) was used as the internal standard and amounts of PLFAs were expressed as equivalent peak responses to the internal standard.

Microscopy. The cellular morphology of B33D1^T was observed by phase-contrast microscopy, negative staining with nigrosin dye and electron microscopy. A Nikon TE 300 inverted microscope was used to observe the cellular morphology of B33D1^T. Digital photomicrographs were taken through the IP Lab Spectrum software package

(version 3.4.5) using an attached Princeton Instruments MicroMax high-resolution, cooled CCD camera. Samples for scanning electron microscopy (SEM) were prepared according to the method of Hahn *et al.* (1998) except that 4% glutaraldehyde was used to fix the cells. A Leo 982 field emission SEM was used to examine the cells. To test for the presence of a capsule, an India ink suspension, as well as a standard capsule stain, were used (Benson, 1990). The Gram reaction of isolate B33D1^T was determined by a common staining technique and observed using light microscopy (Benson, 1990).

Phylogeny. The nearly complete 16S rRNA sequence of B33D1^T was obtained by amplification of the genomic DNA with primers 27f and 1392r (Lane *et al.*, 1991; Furlong *et al.*, 2002). Sequencing reactions were performed using a Big Dye sequencing kit (Perkin-Elmer) with primers 27f, 1392r, 533f, 519r and 907r (Lane, 1991). The sequencing reaction products were run on an ABI 377 Automated Sequencer (Perkin-Elmer). To construct the phylogenetic trees, the sequence was first aligned with reference organisms and environmental clone sequences using the PILEUP program included in the GCG software package. The evolutionary distances between aligned sequences were determined using a Jukes–Cantor algorithm in the DNADIST program of the PHYLIP software package (Felsenstein, 1989). The tree topology was determined using the FITCH program and the tree robustness was testing using 100 replicate trees as generated by SEQBOOT within the PHYLIP set of programs. The nearest phylogenetic neighbours of isolate B33D1^T were determined by FASTA searches (Pearson & Lipman, 1988) of GenBank.

DNA G+C content. The G+C content of isolate B33D1^T was determined by the method of Mesbah *et al.* (1989). The value reported was the mean of five replicates.

RESULTS AND DISCUSSION

Cellular and colonial properties

Cells of strain B33D1^T were rods, approximately 1.4 × 0.7 µm, although much longer cells were occasionally observed in fresh cultures (Fig. 1). Older cultures generally had uniformly shorter rods. In liquid culture, cells were often observed with an indentation near the centre of the cell, presumably due to preparation for cellular division. Also in liquid culture, cells often grew in long chains that wrapped around each other, producing large aggregates. No capsule was observed. Cells of strain B33D1^T stained Gram-positive. No motility was observed and no spores were apparent by phase-contrast microscopy.

Colonies of strain B33D1^T grown on Difco nutrient broth agar plates were round, convex, with entire edges, and usually pink in colour. Plates incubated at higher temperatures (≥28 °C) often initially produced less pigment, although the deep-pink colour appeared over time. Strain B33D1^T had a low growth efficiency upon streaking on agar plates, and only a few or no colonies resulted upon spreading on solid agar surfaces. However, B33D1^T did grow in a liquid culture or embedded in soft, nutrient agar. The low viability of cells on the surface of an agar plate was at least partially explained by their desiccation sensitivity. Strain B33D1^T cells rapidly lost viability upon desiccation when compared with *E. coli* (which has normal sensitivity to desiccation) and *D. radiodurans* (which is desiccation resistant; Fig. 2). Strain B33D1^T was also sensitive to high

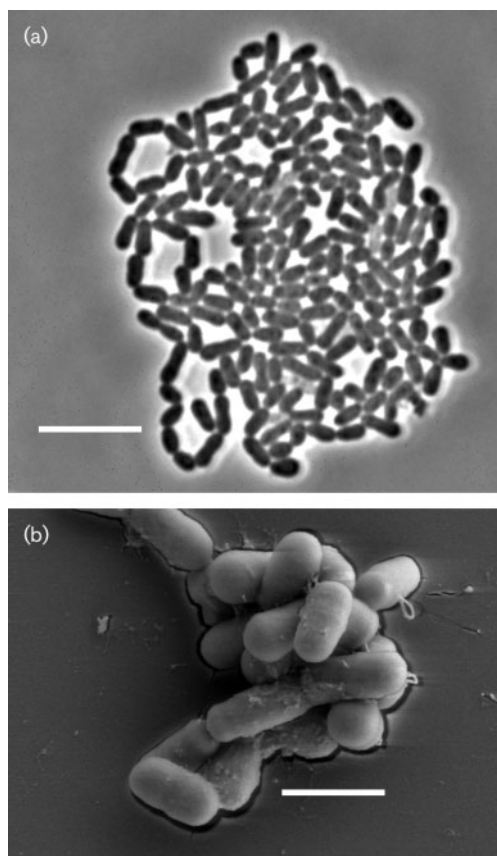


Fig. 1. Photomicrographs of cells of strain B33D1^T grown in NB broth. (a) Phase-contrast image of an aggregate of cells. Bar, 5 μm. (b) SEM of an aggregate. Bar, 1 μm.

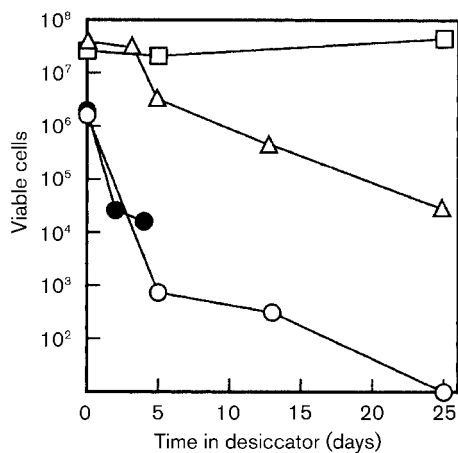


Fig. 2. Effects of desiccation on strain B33D1^T (○, ●), *E. coli* (△) and *D. radiodurans* (□). Multiple lines for strain B33D1^T represent separate experiments.

temperature. Following incubation of a cell suspension containing 10⁶ c.f.u. in PBS at 70 °C for 30 min, no c.f.u. were detected by pour plating. Although phylogenetically related *Rubrobacter* species are resistant to high levels of radiation (Ferreira *et al.*, 1999), the apparent desiccation and heat sensitivity of B33D1^T may indicate poor radiation resistance, as the phenotypes are often correlated (e.g. Billi & Potts, 2002; Mattimore & Battista, 1996; Sanders & Maxcy, 1979).

Strain B33D1^T appeared similar in colour to other *Rubrobacter* organisms. As reported for those organisms, the pigments were not easily extracted using traditional methods. However, a small amount of pigment was recovered by extensive incubation of a cell pellet in methanol. A visible light spectrum of these compounds in 100 % methanol produced maxima at 466, 493 and 526 nm. These maxima are similar to those reported for pigments extracted from *R. radiotolerans* (Saito *et al.*, 1994).

Nutritional characteristics

A variety of compounds were tested as sole carbon and energy sources for strain B33D1^T. Strain B33D1^T generally grew to a low cellular density even in a rich medium, where the maximum OD₆₀₀ was near 0.1. Growth was observed on a variety of sugars, including fructose, galactose, glucose, lactose, mannose, sorbitol, sucrose and xylose. Growth was not observed on cellobiose or mannitol. Strain B33D1^T utilized pyruvate and appeared to grow weakly on acetate, but did not grow on citrate, malate or succinate. Casamino acids provided good growth. When tested individually, only the amino acids alanine, arginine and lysine supported growth. Glycerol was the only alcohol tested that could support the growth of B33D1^T. Other alcohols that did not support growth were methanol, ethanol, 1-propanol, 2-propanol, butanol, isobutyl alcohol and isoamyl alcohol. Because strain B33D1^T was isolated from soil, a variety of products of lignin degradation were tested as possible carbon sources. Of the compounds tested, only chlorogenic acid supported growth. Anthranilic acid, benzoic acid, catechol, protocatechuic acid, *p*-coumaric acid, gentisic acid, ferulic acid, *p*-hydroxybenzoic acid, syringic acid and vanillic acid did not produce observable growth after 2 weeks.

Strain B33D1^T utilized ammonium as a sole nitrogen source and could grow on Casamino acids as a sole source of carbon and nitrogen. It did not reduce nitrate or produce urease.

Other growth conditions

A variety of pH and temperature conditions were tested. Strain B33D1^T grew optimally at 28–30 °C, but could grow at between 19 and 38 °C. Strain B33D1^T could grow at pH 6–7.5, with an optimum around pH 6–6.5. Strain B33D1^T did not grow in the absence of oxygen but grew when 1 % (v/v) air was added to the headspace. Because the concentration of CO₂ is often higher in soil than in the

atmosphere, the growth of strain B33D1^T under these conditions was tested. Increased CO₂ levels (1–5 %, v/v) did not enhance the growth rate or yield significantly. While *Rubrobacter* strains could grow in media with increased salt concentrations (Ferreira *et al.*, 1999; Carreto *et al.*, 1996; Suzuki *et al.*, 1988), strain B33D1^T did not grow when as little as 1 % NaCl was added to the growth medium.

Biochemical and chemotaxonomic properties

Strain B33D1^T was catalase-positive and oxidase-negative. Casein and Tween 80 were not hydrolysed. Strain B33D1^T was sensitive to polymyxin, ampicillin, tetracycline and streptomycin. No haemolysis was observed on blood agar.

The fatty acid profile of B33D1^T revealed no unusual compounds. The major PLFAs were i16:0 (54 %) and 18:1 ω 9c (36 %). Trace amounts of 16:0 (4 %), 19:1 ω 12c (2 %), 16:1 ω 7c (1 %) and br17:1 (1 %) were also detected. No polyunsaturated PLFAs were found. Phylogenetically related *Rubrobacter* isolates contain only small amounts, if any, of the PLFAs that were abundant in strain B33D1^T (Carreto *et al.*, 1996).

The G+C content of the genomic DNA of strain B33D1^T was 71.8 ± 0.2 mol% (mean ± SD; n = 5), which is slightly higher than the value of 67.6 mol% reported for *R. xylanophilus* (Carreto *et al.*, 1996) but not unexpected, given the high G+C content common for members of the phylum *Actinobacteria*.

Phylogeny

Based on 16S rRNA sequence analyses, strain B33D1^T grouped within the subclass *Rubrobacteridae* of the phylum *Actinobacteria*, more specifically within subgroup 2 as defined by Holmes *et al.* (2000) (Fig. 3). No other isolates have been reported from this particular subgroup, although a number of environmental 16S rRNA genes have been described. The most closely related sequence in GenBank by FASTA analysis was an uncultivated clone (YNPFFP1; accession no. AF391984) from a thermal soil (93 % identity over 1364 bases), while the closest characterized organism was *R. xylanophilus* (86 % over 1372 bases). The other cloned sequences in subgroup 2 originated from terrestrial or sediment sources, as did strain B33D1^T. Given the apparently ubiquitous distribution of this group in soil

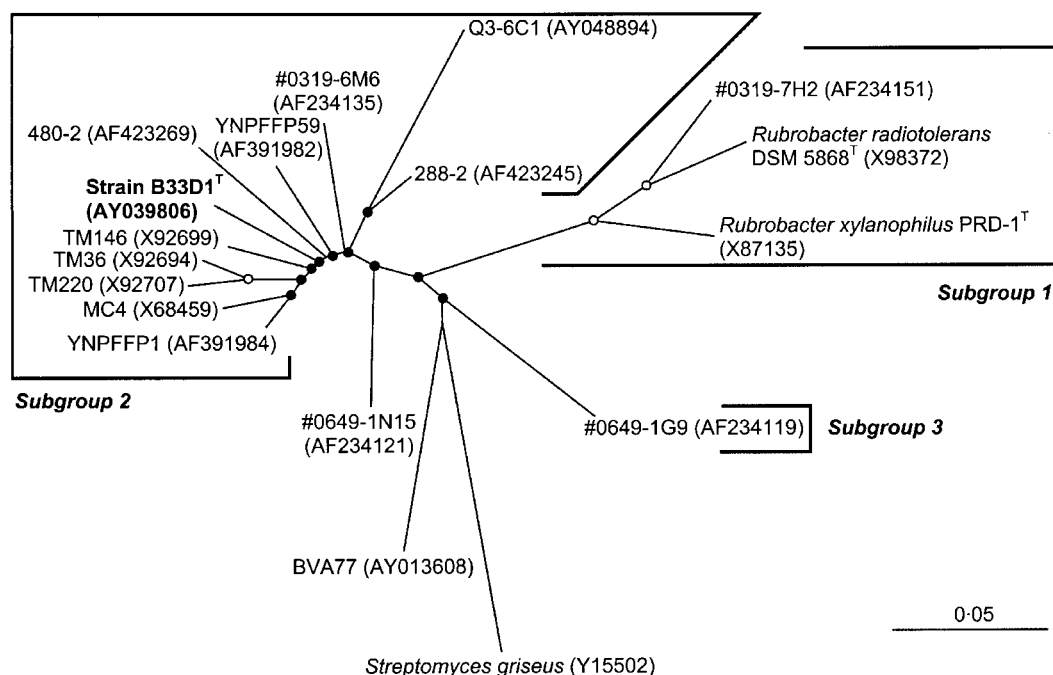


Fig. 3. Phylogenetic tree based on 16S rRNA genes of strain B33D1^T and its closest clonal and cultured relatives. Subgroup designations follow the nomenclature of Holmes *et al.* (2000). Closed and open circles respectively indicate bootstrap support of ≥ 50 and ≥ 95 %. GenBank accession numbers are in parentheses. Only clonal sequences with nearly complete 16S rDNA sequence information were used in this tree. Clones prefixed 'TM' are from a peat bog (Rheims *et al.*, 1996). Clones #0649-1G9, #0319-7H2, #0649-1N15 and #0319-6M6 were obtained from an arid Australian soil (Holmes *et al.*, 2000). Sequences YNPFFP1 and YNPFFP59 were from a thermal soil (L. M. Botero, M. D. Burr, D. Willits, J. G. Elkins, W. P. Inskeep and T. R. McDermott, unpublished). Clone MC4 was from a subtropical Australian soil (Liesack & Stackebrandt, 1992). Clones 480-2 and 288-2 were from soil (Valinsky *et al.*, 2002). BVA77 and Q3-6C1 were respectively from a landfill (Röling *et al.*, 2001) and rhizosphere soil (Heuer *et al.*, 2002). The tree was based on 1306 bases of aligned sequence. The scale bar represents Jukes–Cantor evolutionary distance.

Table 1. Comparison of selected properties of strain B33D1^T with those of *Rubrobacter* type strains

Data for reference species were taken from Carreto *et al.* (1996), Suzuki *et al.* (1988) and Yoshinaka *et al.* (1973).

Property	Strain B33D1 ^T	<i>R. radiotolerans</i> JCM 2153 ^T	<i>R. xylanophilus</i> PRD-1 ^T
Cell size (µm)	1.4 × 0.7	1–4 × 0.8–1.0	1–3 × 0.9–1.0
Temperature optimum (°C)	28–30	46–48	60
pH optimum	6.0–6.5	7.0–7.4	7.5–8.0
DNA G+C content (mol%)	71.8	67.9	67.7
Major fatty acid	i16:0	12-methyl-16:0	14-methyl-18:0
Growth in NaCl	<1%	≤6%	≤6%
Sorbitol utilization	+	–	–
Oxidase production	–	+	+

samples in various parts of the world, there is little reason to assume an association of this organism with earthworms, even though it was originally isolated from earthworm burrow soil.

Because of its low relatedness to previously described organisms, strain B33D1^T appears to represent a novel genus and species. This conclusion is supported by phenotypic differences from representatives of the most closely related taxa (Table 1). The lower temperature optimum and sensitivity to NaCl appear to be the most important distinguishing features. Lastly, the poor plating efficiency and sensitivity to desiccation may explain why this taxon is so poorly represented in culture collections. Presumably, more representatives might be easily isolated from soil by pour plating.

Description of *Solirubrobacter* gen. nov.

Solirubrobacter (So.li.ru.bro.bac' ter. L. n. *solum* soil; N.L. n. *Rubrobacter* a bacterial genus; N.L. masc. n. *Solirubrobacter* a *Rubrobacter*-like bacterium from soil).

Cells are Gram-positive rods of medium length. Non-motile. Spores are not formed. Aerobic and mesophilic. Catalase-positive and oxidase-negative. Grow well on common sugars and a few amino acids as sole carbon sources. Sensitive to desiccation. Phylogenetically, placed within the subclass *Rubrobacteridae* of the *Actinobacteria*. The type species is *Solirubrobacter pauli*.

Description of *Solirubrobacter pauli* gen. nov., sp. nov.

Solirubrobacter pauli (pau'li. L. gen. n. *pauli* of *Paulus*, named for the prominent soil microbiologist Eldor A. Paul).

Cells are rods, 1.4 × 0.7 µm, and grow in long chains. Colonies are round, convex and pink in colour. The G+C content of the type strain is 71.8 mol%. The major fatty acids are i16:0 (54%) and 18:1ω9c (36%). The type strain grows at pH 6.0–7.5 and 19–38 °C, with optima at pH 6.5 and 28–30 °C. Fructose, galactose, glucose, lactose, mannose, sorbitol, sucrose, xylose, pyruvate, acetate, Casamino acids, alanine, arginine, lysine, glycerol and chlorogenic acid

support growth. Does not grow on cellobiose, mannitol, citrate, malate, succinate, methanol, ethanol, 1-propanol, 2-propanol, butanol, isobutyl alcohol, isoamyl alcohol, anthranilic acid, benzoic acid, catechol, protocatechuic acid, *p*-coumaric acid, gentisic acid, ferulic acid, *p*-hydroxybenzoic acid, syringic acid or vanillic acid. The type strain is strain B33D1^T (=ATCC BAA-492^T =DSM 14954^T).

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REFERENCES

- Balch, W. E., Fox, G. E., Magrum, L. J., Woese, C. R. & Wolfe, R. S. (1979). Methanogens: reevaluation of a unique biological group. *Microbiol Rev* **43**, 260–296.
- Benson, H. J. (1990). *Microbiological Applications: a Laboratory Manual in General Microbiology*. Dubuque, IA: Wm. C. Brown.
- Billi, D. & Potts, M. (2002). Life and death of dried prokaryotes. *Res Microbiol* **153**, 7–12.
- Bligh, E. G. & Dyer, W. J. (1954). A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
- Carreto, L., Moore, E., Nobre, M. F., Wait, R., Riley, P. W., Sharp, R. J. & da Costa, M. S. (1996). *Rubrobacter xylanophilus* sp. nov., a new thermophilic species isolated from a thermally polluted effluent. *Int J Syst Bacteriol* **46**, 460–465.
- Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Ferreira, A. C., Nobre, M. F., Moore, E., Rainey, F. A., Battista, J. R. & da Costa, M. S. (1999). Characterization and radiation resistance of new isolates of *Rubrobacter radiotolerans* and *Rubrobacter xylanophilus*. *Extremophiles* **3**, 235–238.
- Furlong, M. A., Singleton, D. R., Coleman, D. C. & Whitman, W. B. (2002). Molecular and culture-based analyses of prokaryotic communities from an agricultural soil and the burrows and casts of the earthworm *Lumbricus rubellus*. *Appl Environ Microbiol* **68**, 1265–1279.

- Guckert, J. B., Antworth, C. P., Nichols, P. D. & White, D. C. (1985).** Phospholipid ester-linked fatty acid profiles as reproducible assays for changes in prokaryotic community structure of estuarine sediments. *FEMS Microbiol Ecol (FEMS Microbiol Lett Special Issue)* **31**, 147–158.
- Hahn, T.-W., Willby, M. J. & Krause, D. C. (1998).** HMW1 is required for cytoadhesion P1 trafficking to the attachment organelle in *Mycoplasma pneumoniae*. *J Bacteriol* **180**, 1270–1276.
- Heuer, H., Kroppenstedt, R. M., Lottmann, J., Berg, G. & Smalla, K. (2002).** Effects of T4 lysozyme release from transgenic potato roots on bacterial rhizosphere communities are negligible relative to natural factors. *Appl Environ Microbiol* **68**, 1325–1335.
- Holmes, A. J., Bowyer, J., Holley, M. P., O'Donoghue, M., Montgomery, M. & Gillings, M. R. (2000).** Diverse, yet-to-be-cultured members of the *Rubrobacter* subdivision of the Actinobacteria are widespread in Australian arid soils. *FEMS Microbiol Ecol* **33**, 111–120.
- Lane, D. J. (1991).** 16S/23S rRNA sequencing. In *Nucleic Acid Sequencing Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. New York: Wiley.
- Liesack, W. & Stackebrandt, E. (1992).** Occurrence of novel groups of the domain *Bacteria* as revealed by analysis of genetic material isolated from an Australian terrestrial environment. *J Bacteriol* **174**, 5072–5078.
- Mattimore, V. & Battista, J. R. (1996).** Radioresistance of *Deinococcus radiodurans*: functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. *J Bacteriol* **178**, 633–637.
- McCaig, A. E., Glover, L. A. & Prosser, J. I. (1999).** Molecular analysis of bacterial community structure and diversity in unimproved and improved upland grass pastures. *Appl Environ Microbiol* **65**, 1721–1730.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989).** Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Pearson, W. R. & Lipman, D. J. (1988).** Improved tools for biological sequence comparison. *Proc Natl Acad Sci U S A* **85**, 2444–2448.
- Rheims, H., Spröer, C., Rainey, F. A. & Stackebrandt, E. (1996).** Molecular biological evidence for the occurrence of uncultured members of the actinomycete line of descent in different environments and geographical locations. *Microbiology* **142**, 2863–2870.
- Röling, W. F. M., van Breukelen, B. M., Braster, M., Lin, B. & van Verseveld, H. W. (2001).** Relationships between microbial community structure and hydrochemistry in a landfill leachate-polluted aquifer. *Appl Environ Microbiol* **67**, 4619–4629.
- Saito, T., Terato, H. & Yamamoto, O. (1994).** Pigments of *Rubrobacter radiotolerans*. *Arch Microbiol* **162**, 414–421.
- Sanders, S. W. & Maxcy, R. B. (1979).** Isolation of radiation-resistant bacteria without exposure to irradiation. *Appl Environ Microbiol* **38**, 436–439.
- Suzuki, K., Collins, M. D., Iijima, E. & Komagata, K. (1988).** Chemotaxonomic characterization of a radiotolerant bacterium *Arthrobacter radiotolerans*: description of *Rubrobacter radiotolerans* gen. nov., comb. nov. *FEMS Microbiol Lett* **52**, 33–40.
- Ueda, T., Suga, Y. & Matsuguchi, T. (1995).** Molecular phylogenetic analysis of a soil microbial community in a soybean field. *Eur J Soil Sci* **46**, 415–421.
- Valinsky, L., Della Vedova, G., Scupham, A. J. & 8 other authors (2002).** Analysis of bacterial community composition by oligonucleotide fingerprinting of rRNA genes. *Appl Environ Microbiol* **68**, 3243–3250.
- White, D. C., Davis, W. M., Nickels, J. S., King, J. D. & Bobbie, R. J. (1979).** Determination of the sedimentary microbial biomass by extractable lipid phosphate. *Oecologia* **40**, 51–62.
- Whitman, W. B., Shieh, J., Sohn, S., Caras, D. S. & Premachandran, U. (1986).** Isolation and characterization of 22 mesophilic methanococci. *Syst Appl Microbiol* **7**, 235–240.
- Yoshinaka, T., Yano, K. & Yamaguchi, H. (1973).** Isolation of highly radioresistant bacterium, *Arthrobacter radiotolerans* nov. sp. *Agric Biol Chem* **37**, 2269–2275.