**Nocardioides oleivorans** sp. nov., a novel crude-oil-degrading bacterium

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The crude-oil-degrading strain BAS3ᵀ represents a novel *Nocardioides* species, according to a taxonomic study. The 16S rRNA gene sequence of strain BAS3ᵀ was most similar to that of *Nocardioides ganghvensis* (IMSNU 14028ᵀ; 99 % similarity), but the DNA–DNA relatedness to this type strain was only 32 %. The physiological properties of strain BAS3ᵀ differ from those of *N. ganghvensis* (IMSNU 14028ᵀ) and other species of *Nocardioides*. The diamino acid in the cell-wall peptidoglycan of strain BAS3ᵀ is LL-diaminopimelic acid and the major menaquinone is MK-8(H₄). The name *Nocardioides oleivorans* sp. nov. is proposed for the novel *Nocardioides* species, since its type strain, BAS3ᵀ (≡ DSM 16090ᵀ = NCIMB 14004ᵀ), is able to degrade crude oil.

Crude oil consists of various hydrocarbons that can be degraded by micro-organisms (Jobson et al., 1972; Bosecker et al., 1991; Zengler et al., 1997). Several genera of hydrocarbon-oxidizing bacteria are known (Rosenberg, 2000; Van Hamme et al., 2003). The aromatic-compound-degrading species of the genus *Nocardioides* comprise the pyridine-degrading species *Nocardioides pyridinoliticus* (Yoon et al., 1997), the p-nitrophenol-degrading species *Nocardioides nitrophenolicus* (Yoon et al., 1999) and the pyric acid (2,4,6-trinitrophenol)-degrading *Nocardioides* species strain CB 22-2 (Rajan et al., 1996; Behrendt & Heesche-Wagner, 1999).

Here we describe the classification of the crude-oil-degrading strain BAS3ᵀ as a member of the genus *Nocardioides*. The Gram-positive strain was isolated from a crude oil sample 19 from the oilfield Oerrel of the Gifhorn Trough, North-West Germany (Bosecker et al., 1991). For enrichment, artificial sea-water medium, supplemented with 1–5 % (w/v) crude oil as the carbon source, in Erlenmeyer flasks was inoculated and incubated on a rotary shaker at 30 °C in the dark for several weeks. The medium (pH 7·3) consisted of the following (l⁻¹): 23·4 g NaCl, 0·75 g KCl, 7·0 g MgSO₄.7H₂O, 1·0 g NH₄NO₃, 0·7 g K₂HPO₄ and 0·3 g KH₂PO₄ (Fedorak & Westlake, 1981).

For isolation via subculturing on agar plates, a basal medium (pH 7·3), without oil, was used, consisting of the following (l⁻¹): 23·4 g NaCl, 0·75 g KCl, 7·0 g MgSO₄.7H₂O, 0·5 g peptone from meat, 0·5 g peptone from casein, 1·0 g yeast extract and 18 g agar.

The morphology of the cells, their motility and the occurrence of spores were investigated by using phase-contrast light microscopy (Axioskop microscope; Zeiss). A Gram-stain and a test for catalase were performed according to Burghardt (1992) and Gerhardt et al. (1994). Anaerobic growth was checked by means of incubation in the presence and absence of oxygen, using the AnaeroCult system (Merck). Further physiological tests were carried out as described by Kämpfer et al. (1991). Briefly, the utilization of various carbon sources as sole substrate and the hydrolysis of various compounds were studied using a complex medium containing trace elements and vitamins in microplates. To confirm oil degradation, 40 ml medium (Kämpfer et al., 1991; modified) in 100 ml Erlenmeyer flasks was supplemented with 1 ml crude oil as the sole carbon source, inoculated and then incubated on a rotary shaker (120 r.p.m.) at 25 °C. Cell growth was checked after 3 weeks. The modified medium had a pH of 7·0 and the following composition (l⁻¹): 1·0 g NaCl, 0·1 g MgSO₄.7H₂O, 1·0 g (NH₄)₂SO₄, 3·2 g K₂HPO₄, 6·18 g Na₂HPO₄.2H₂O, 0·17 g CaSO₄.2H₂O, 0·001 g H₃BO₃, 0·002 g CuSO₄.5H₂O, 0·003 g ZnCl₂.8H₂O, 0·2 g Fe₂(SO₄).7H₂O, 0·002 g NiCl₂.6H₂O, 0·004 g CoCl₂.6H₂O, 0·01 g MnCl₂.5H₂O, 0·003 g Na₂MoO₄.2H₂O, 0·5 g EDTA.2H₂O and vitamins according to Kämpfer et al. (1991).

The occurrence of diaminopimelic acid in the cell wall and the whole-cell fatty acid composition were determined using the MIDI Sherlock System (MIDI, USA) according to the MIDI standard (1987).
also the peptidoglycan type were determined as described by Schleifer (1985) and Schleifer & Kandler (1972), using TLC with cellulose plates (Merck). Menaquinones were extracted as described by Collins et al. (1977) and were analysed by HPLC according to Groth et al. (1996). Analysis of the whole-cell fatty acid pattern of cells grown on the basal medium was performed with the MIDI system (Microbial ID), using previously described methods (Kroppenstedt, 1985; Meier et al., 1993).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and purification of PCR products were carried out as described previously (Rainey et al., 1996). Purified PCR products were sequenced with Taq DyeDeoxy terminator cycle sequencing kits (Applied Biosystems) according to the manufacturer’s protocol. An Applied Biosystems 373A DNA sequencer was used for electrophoresis of the sequence reaction products. The ae2 editor (Maidak et al., 1999) was used to align the 16S rRNA gene sequence determined in this study against the 16S rRNA gene sequences (available from the public databases) of representatives of the main bacterial lineages. Pairwise evolutionary distances were computed using the correction of Jukes & Cantor (1969). The least-squares distance evolution of species and species distances were computed using the construction of the phylogenetic dendrogram from distance matrices. Bootstrap analyses were done as described by Felsenstein (1993).

For DNA–DNA reassociation experiments, DNA was isolated using a French pressure cell (Thermo Spectronic) and purified by chromatography on hydroxyapatite, as described by Cashion et al. (1977). DNA–DNA hybridization was carried out under optimal conditions for DNA–DNA reassociation as described by De Ley et al. (1970), with the modifications described by Huß et al. (1983), using a Cary 100 Bio UV/VIS spectrophotometer equipped with a Peltier thermostatted 6 × 6 multicliff cell and a temperature controller with an in situ temperature probe (Varian).

The almost-complete 16S rRNA gene sequence of strain BAS3T was compared with those of members of closely related genera. Members of the genus Nocardioides were the closest phylogenetic neighbours. A maximum pairwise similarity value of 99% was found for Nocardioides ganghwensis IMSNU 14028T. Pairwise similarity values higher than 95% were also found for Nocardioides aquitae KCCM 41647T (95.4%), N. pyriddinolyticus KCTC 0074BP3 (95.2%), Nocardioides simplex KCTC 9106T (95.2%) and N. nitrophilicus KCTC 0457BP3 (95.1%); a phylogenetic tree of the Nocardioides species and species of closely related genera is available as supplementary material in IJSEM Online.

Because of the high pairwise similarity value (99%) between strain BAS3T and N. ganghwensis, the DNA–DNA relatedness of these two strains was determined. The value obtained was 32%, which is well below the threshold value of 70% recommended for the definition of bacterial species (Wayne et al., 1987).

The morphological, physiological and chemotaxonomic characteristics of strain BAS3T were analysed. The properties are given in the description section. Several physiological properties of strain BAS3T and the closest phylogenetic neighbour, N. ganghwensis IMSNU 14028T (Yi & Chun, 2004), were compared. For the latter strain, oil degradation could not be observed, and the following carbon sources were utilized by this strain only: citrate, salicin and L-arabinose. L-Rhamnose, however, was utilized only by strain BAS3T. Both strains utilized D-cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, L-ornithine and N-acetylg glucosamine.

The fatty acid profile of strain BAS3T was characteristic of members of the genus Nocardioides (iso-C16:0 as the branched fatty acid plus an abundance of 10-methyl fatty acids). The major fatty acids of strain BAS3T were C18:1ω9C, iso-C16:0, C18:0 and 10-methyl fatty acids (the whole-cell fatty acid composition of strain BAS3 and those of closely related Nocardioides type strains are available as a supplementary table in IJSEM Online). Strain BAS3T contained LL-diaminopimelic acid in the cell wall and had A3γ-type peptidoglycan (LL-diaminopimelic acid–Gly). MK-8(H4) was the major menaquinone and MK-8(H2) was a minor menaquinone component.

On the basis of the polyphasic evidence, we suggest that strain BAS3T represents a novel species, for which we propose the name Nocardioides oleivorans sp. nov.

**Description of Nocardioides oleivorans sp. nov.**

*Nocardioides oleivorans* [o.ле.i.vор’ans. L. n. *oleum* oil; L. v. *vorare* to devour; N.L. part. adj. *oleivorans* capable of utilizing oil (hydrocarbons)].

Cells are obligate aerobic, Gram-positive, non-endospore-forming, non-motile, irregular rods about 0.3 μm wide and up to 1.1 μm long. Catalase-positive and oxidase-negative. Colonies are circular, smooth, translucent and orange-pigmented with a maximum colony diameter of 2 mm after 2 weeks. Growth occurs at 30°C and with 2% (w/v) NaCl. Crude oil is used as substrate. Acid is not produced from D-glucose, rhamnose, sucrose, adonitol, inositol, xylose or sorbitol. Utilization of N-acetyl-D-glucosamine, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-maltose, D-mannose, α-D-melibiose, L-rhamnose, D-sucrose, D-trehalose, D-mannitol, acetate, propionate, fumarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, pyruvate, L-aspartate, L-histidine, L-proline, putrescine, phenylacetate and L-ornithine is observed. L-Arabinose, α-D-galactonate, glycerol, D-ribose, salicin, L-xylose, adonitol, i-inositol, sorbitol, *trans*-aconitate, adipate, citrate, suberate, L-alanine, L-hydroxyproline, L-serine, 3-hydroxybenzoate, 4-hydroxybenzoate and N-acetyl-D-galactosamine are not

The type strain is strain BASS/T (= DSM 16090 = NCIMB 14004T), which was isolated from a crude oil sample from the oilfield Oerrel of the Gifhorn Trough, North-West Germany (Bosecker et al., 1991).

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