Nocardia neocaledoniensis sp. nov., a novel actinomycete isolated from a New-Caledonian brown hypermagnesian ultramafic soil

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The taxonomic position of an actinomycete isolated from a hypermagnesian ultramafic soil was examined using a polyphasic approach. The strain, designated SBH_R OA6^T, was shown to have chemical and morphological properties typical of members of the genus *Nocardia*. The organism was most closely associated with *Nocardia asteroides* using 16S rRNA gene sequence data. It showed a distinctive set of phenotypic properties that distinguished it from representatives of all species with validly published names classified in the genus *Nocardia*. The combined genotypic and phenotypic data show that strain SBH_R OA6^T (=DSM 44717^T=NCIMB 13955^T) merits description as the type strain of a novel *Nocardia* species, *Nocardia neocaledoniensis* sp. nov.

The genus *Nocardia* is well defined, due mainly to the application of chemotaxonomic, molecular systematic and numerical phenetic methods (Goodfellow *et al.*, 1999). At the time of writing, the taxon contains 29 species with validly published names that form a clade within the evolutionary radiation occupied by mycolic-acid-containing actinomycetes classified in the suborder *Corynebacterineae* Stackebrandt *et al.* 1997. The genus can be distinguished from the other genera assigned to the suborder by a combination of chemical and morphological properties (Goodfellow *et al.*, 1999). Similarly, *Nocardia* species can be separated from one another by using a range of phenotypic properties (Yassin *et al.*, 2001; Zhang *et al.*, 2003). The improved classification of the genus is providing an invaluable framework for the recognition of novel *Nocardia* species, as exemplified by the description of novel species of clinical (Yassin *et al.*, 2000; Hamid *et al.*, 2001), ecological (Chun *et al.*, 1998; Maldonado *et al.*, 2000; Albuquerque de Barros *et al.*, 2003) and industrial (Isik *et al.*, 1999b; Kinoshita *et al.*, 2001) significance.

Ultramafic soils account for about a third of the landmass of the main island of New Caledonia (Jaffré, 1976). There is evidence that these arid, infertile soils, which have high metal toxicity due to the presence of chromium, cobalt, iron and nickel, provide a rich habitat for unusual actinomycetes of industrial significance (Saintpierre, 2001; Saintpierre *et al.*, 2003; D. Saintpierre-Bonaccio, H. Amir, R. Pineau and M. Goodfellow, unpublished results). During the course of a screening programme designed to isolate novel actinomycetes from New-Caledonian ultramafic soils, an actinomycete, designated SBH_R OA6^T, was isolated and presumptively assigned to the genus *Nocardia* (Saintpierre, 2001). The aim of the present study was to establish the taxonomic position of this organism using genotypic and phenotypic procedures. It is evident from the results that the organism represents a novel species in the genus *Nocardia*, for which the name *Nocardia neocaledoniensis* sp. nov. is proposed.

Strain SBH_R OA6^T was isolated from a suspension of a brown hypermagnesian ultramafic soil that was used to inoculate an oatmeal agar plate (ISP 3 medium; Shirling & Gottlieb, 1966) supplemented with cycloheximide (100 μg ml^{-1}) and incubated at 30 °C for 10 days. The soil sample was from the 'Plum' region at the southern end of the main island of New Caledonia [see Institut National Geographique, map no. 38 (Mont Dore), 669 × 7536·5, série orange]. The isolate was purified and maintained on modified Bennett's agar (MBA; Jones, 1949) and preserved as a suspension of mycelial fragments in glycerol (20 %, v/v) at −20 °C. Biomass for chemotaxonomic studies was prepared by growing the strain in shake flasks of glucose/yeast extract broth (Gordon & Mihm, 1962) at 100 r.p.m. for 10 days at 30 °C. Cultures were checked for purity,

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Abbreviation: MBA, modified Bennett's agar.

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killed by shaking with formalin (1 %, v/v), harvested by centrifugation and freeze-dried.

Morphological and staining properties of strain SBH_R OA6^T were detected following growth on MBA plates that had been incubated for 2 weeks at 30 °C. Additional phenotypic properties were determined using well-established methods (Williams *et al.*, 1983; Isik *et al.*, 1999a). Standard procedures were used for the extraction and analysis of mycolic acids (Minnikin *et al.*, 1975), whole-organism sugars (Schaal, 1985) and isoprenoid quinones and polar lipids (Minnikin *et al.*, 1984), using appropriate controls. The isomeric form of diaminopimelic acid (A_2pm) was determined after Staneck & Roberts (1974), using a modified solvent system consisting of methanol/water/10 M HCl/pyridine (85 : 15 : 5 : 10, by vol.).

Biomass for 16S rDNA nucleotide sequencing was obtained by growing strain SBH_R OA6^T on an MBA plate for 7 days at 30 °C. Isolation of chromosomal DNA, PCR amplification and direct sequencing of the purified product were carried out as described previously (Kim *et al.*, 1999). The resultant 16S rRNA gene sequence was aligned manually with corresponding sequences of representatives of the genera classified in the suborder *Corynebacterineae* retrieved from the DDBJ/EMBL/GenBank databases using the PHYDIT program (Chun, 1995). Evolutionary trees were inferred using the least-squares (Fitch & Margoliash, 1967), maximum-parsimony (Kluge & Farris, 1969) and neighbour-joining (Saitou & Nei, 1987) treeing algorithms from the PHYLIP suite of programs (Felsenstein, 1993). Evolutionary-distance matrices for the least-squares and neighbour-joining methods were generated after Jukes & Cantor (1969). The topologies of the resultant trees were evaluated by bootstrap analyses (Felsenstein, 1985) of the neighbour-joining datasets on 1000 resamplings using the SEQBOOT and CONSENSE options from the PHYLIP package. DNA–DNA relatedness values between strain SBH_R OA6^T and related strains were determined by the identification service at the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany), as described by Kim *et al.* (1999).

Strain SBH_R OA6^T has phenotypic properties consistent with its classification in the genus *Nocardia* (Goodfellow *et al.*, 1999). The organism is an aerobic, Gram-positive, partially acid–alcohol-fast actinomycete that forms an extensively branched substrate mycelium that fragments into irregular, rod-shaped, non-motile elements and supports pale-orange aerial hyphae when grown on glucose/yeast extract agar and MBA plates. The organism was also shown to yield whole-organism hydrolysates rich in *meso*-A_2pm, arabinose and galactose (wall chemotype IV *sensu* Lechevalier & Lechevalier, 1970) and to have major proportions of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides (phospholipid type II *sensu* Lechevalier *et al.*, 1977). It also contained predominant amounts of hexahydrogenated menaquinones with eight isoprene units where the end two were cyclized; this menaquinone is characteristic of the genera *Nocardia* and *Skermania* (Chun *et al.*, 1997; Goodfellow *et al.*, 1999). It is also characterized by the presence of mycolic acids that co-migrated (R_f value around 0·47) with those from marker strains of *Nocardia*.

The almost complete 16S rRNA gene sequence (1474 nt) obtained for strain SBH_R OA6^T was compared with sequences of representatives of the suborder *Corynebacterineae* and found to have the signature sequences expected for members of this taxon and the family *Nocardiaceae* (Stackebrandt *et al.*, 1997). The high 16S rRNA gene sequence similarities found between the tested strain and representatives of the genus *Nocardia* (95·4–98·7 %) support its inclusion in this taxon. The 16S rRNA gene sequence of strain SBH_R OA6^T also has the signature nucleotides characteristic of the genus *Nocardia* (Chun & Goodfellow, 1995).

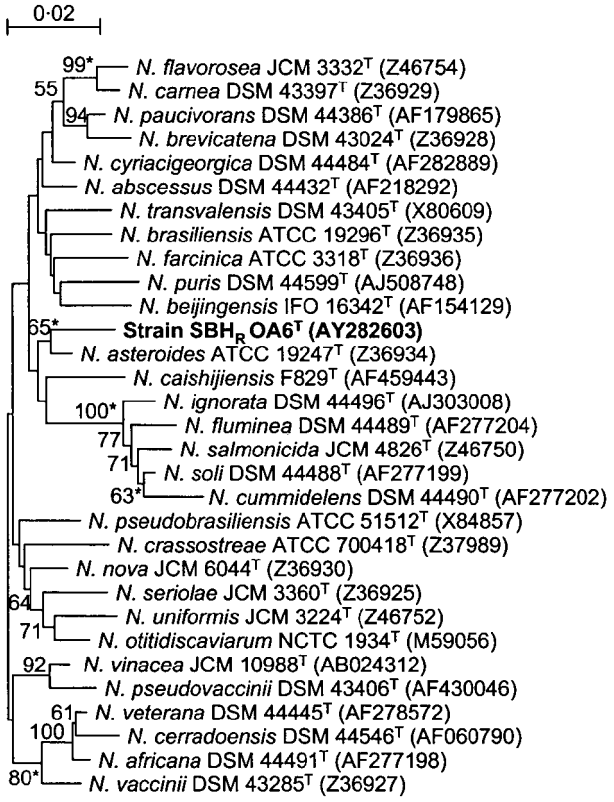

Fig. 1. Neighbour-joining tree (Saitou & Nei, 1987) based on nearly complete 16S rRNA gene sequences showing relationships between strain SBH_R OA6^T and representatives of *Nocardia* species. Asterisks indicate branches of the tree that were also found using the least-squares (Fitch & Margoliash, 1967) and maximum-parsimony (Kluge & Farris, 1969) treeing algorithms. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 50 % are shown. Bar, 0·02 substitutions per site.

It is evident from Fig. 1 that strain SBH_R OA6^T is most closely associated with the type strain of *Nocardia asteroides*, a relationship that is supported by all of the treeing algorithms and by a moderately high bootstrap value. The two strains share 98·7 % 16S rRNA gene sequence similarity, a value that corresponds to 17 nt differences at 1470 locations. However, it is evident from the DNA–DNA relatedness study that the two strains should not be classified in the same species, as they were found to share 40·8 % DNA–DNA relatedness, a value well below the 70 % cut-off point recommended for the delineation of genomic species (Wayne *et al.*, 1987). The organism also has a profile of phenotypic properties that distinguish it from representatives of all species of *Nocardia* with validly published names, including *N. asteroides* (Table 1).

The genotypic and phenotypic data indicate that strain SBH_R OA6^T merits recognition as a novel species of *Nocardia*. It is proposed that the organism be classified in the genus *Nocardia* as *Nocardia neocaledoniensis* sp. nov.

Description of *Nocardia neocaledoniensis* sp. nov.

Nocardia neocaledoniensis (ne.o.ca.le.do.ni.en′sis. N.L. fem. adj. *neocaledoniensis* pertaining to New Caledonia, the source of the isolate).

Aerobic, Gram-positive, catalase-positive, slightly acid–alcohol-fast, non-motile actinomycete that forms an extensively branched orange substrate mycelium that fragments *in situ* into irregular rod-shaped elements and which carries abundant pale-orange aerial hyphae on MBA. Melanin pigments are produced on peptone/yeast extract/iron agar. Chemotaxonomic properties are typical of *Nocardia*. Grows at 10–45 °C and from pH 4 to 12. Degrades DNA and Tween 80 but not gelatin, guanine, starch or xylan. Produces hydrogen sulphide. Utilizes (+)-D-fructose, (+)-D-mannose, (+)-D-raffinose and (+)-D-trehalose as sole carbon sources for energy and growth but not adonitol, (−)-D-arabinose, (+)-D-cellobiose, (+)-D-galactose, (+)-D-lactose, (+)-D-maltose, (+)-D-melibiose, (+)-D-sucrose (all at 1 %, w/v) or sodium

Table 1. Phenotypic properties that distinguish strain SBH_R OA6^T from the type strains of *Nocardia* species

Strains: 1, strain SBH_R OA6^T; 2, *N. abscessus* DSM 44432^T; 3, *N. africana* DSM 44491^T; 4, *N. asteroides* ATCC 19247^T; 5, *N. beijingensis* IFO 16342^T; 6, *N. brasiliensis* ATCC 19296^T; 7, *N. brevicatena* DSM 43024^T; 8, *N. caishijiensis* JCM 11508^T; 9, *N. carnea* DSM 43397^T; 10, *N. cerradoensis* DSM 44546^T; 11, *N. crassostreae* ATCC 700418^T; 12, *N. cummidelens* DSM 44490^T; 13, *N. cyriacigeorgica* DSM 44484^T; 14, *N. farcinica* ATCC 3318^T; 15, *N. flavorosea* JCM 3332^T; 16, *N. fluminea* DSM 44489^T; 17, *N. ignorata* DSM 44496^T; 18, *N. nova* JCM 6044^T; 19, *N. otitidiscaviarum* NCTC 1934^T; 20, *N. paucivorans* DSM 44386^T; 21, *N. pseudobrasiliensis* ATCC 51512^T; 22, *N. puris* DSM 44599^T; 23, *N. salmonicida* JCM 4826^T; 24, *N. seriolae* JCM 3360^T; 25, *N. soli* DSM 44488^T; 26, *N. transvalensis* DSM 43405^T; 27, *N. uniformis* JCM 3224^T; 28, *N. vaccinii* DSM 43285^T; 29, *N. veterana* DSM 44445^T; 30, *N. vinacea* JCM 10988^T. ND, Not determined; D, doubtful. Data for reference strains were taken from Albuquerque de Barros *et al.* (2003), Yassin *et al.* (2003) and Zhang *et al.* (2003) unless indicated otherwise. All strains are negative for decomposition of 0·4 % (w/v) adenine (data for *N. cummidelens* DSM 44490^T from this study).

Property	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Aesculin hydrolysis	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	D	D
Nitrate reduction	+	+	+	+	+	+	−	+	+	+	ND	+	+*	+	−	+	+*	+	+	−	−*	ND	+	+	+	+	+	+	−	+
Urea hydrolysis	+	+	−	+	+	+	−	−	−	+	−	+	+	+	−	+	+*	+	+	+	+	+	+	−	+	+	+	+	+	+
Decomposition of (%, w/v):																														
Casein (1·0)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−
Elastin (0·3)	−	−	−	−	−	+	−	−	−	−	−*	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	+	−	−	−
Hypoxanthine (0·4)	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	+	+	−	−	+
Tyrosine (0·5)	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	+	−	−	−	+	−	−	−
Uric acid (0·5)	−	−	−	−	−	−	−	−	−	−	−	−	ND	−	−	−	ND	−	+	−*	+	ND	−	−	−	+	+	−	−	+
Xanthine (0·4)	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	+	−	−	−
Growth on carbon sources (%, w/v):																														
(+)-D-Mannitol (1·0)	+	−	−	−	+	+	−	−	+	−	−	−	−	−	+	−	+	+	+	−	+	+	+	−	−	+	−	+	−	+
α-L-Rhamnose (1·0)	−	+	−	−	+	−	+	+	−	+	+	−	−	+	−	+	−	−	−	−	−	+	−	−	+	+	−	+	+	−
(+)-D-Sorbitol (1·0)	−	−	−	−	+	−	−	−	+	+	−	−	−	−	+	−	ND	+	−	−	+	+	+	−	−	+	−	−	−	+
(+)-D-Xylose (1·0)	−	−	−	−	+	−	ND	+	−	−	−	+	−	−	ND	+	−	−	−	−	+	−	−	ND	+	−	−	+	ND	ND
Sodium acetate (0·1)	−	+	−	+	+	+	+	−	+	+	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	−	−	ND
Sodium citrate (0·1)	−	+	−	+	+	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	+	+	+	+	−	−	−	−	−	D
Growth at 45 °C	+	−*	+	−	−	+	−	−	−	−	−	−	−	+	−	−	+	−	+	+	−	+	−	−	−	−	−	−	+	−

*Data from this study.

succinate (0·1 %, w/v). Growth occurs in the presence of erythromycin (4 μg ml^{-1}), gentamicin sulphate (10 μg ml^{-1}), penicillin G (25 μg ml^{-1}), rifampicin (6 μg ml^{-1}), streptomycin sulphate (5 μg ml^{-1}), vancomycin hydrochloride (10 μg ml^{-1}), crystal violet (0·0002 %, w/v), phenol (0·01 %, w/v) and sodium chloride (3 %, w/v), but not in the presence of tetracycline hydrochloride (30 μg ml^{-1}), potassium tellurite (0·005 %, w/v) or 5 % (w/v) sodium chloride. Additional phenotypic properties are shown in Table 1. The species description is based on a single strain, which hence serves as the type strain.

The type strain, SBH_R $OA6^T$ (=DSM 44717^T=NCIMB 13955^T), was isolated from a brown hypermagnesian ultramafic soil at the southern end of the main island of New Caledonia.

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