Nocardiosis potens sp. nov., isolated from household waste

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The taxonomic position of an actinomycete, designated strain IMMIB L-21^T, was determined using a polyphasic taxonomic approach. The organism, which had phenotypic properties consistent with its classification in the genus *Nocardiosis*, formed a distinct clade in the 16S rRNA gene sequence tree together with the type strain of *Nocardiosis composta*, but was readily distinguished from this species using DNA–DNA relatedness and phenotypic data. The genotypic and phenotypic data show that the organism represents a novel species of the genus *Nocardiosis*, for which the name *Nocardiosis potens* sp. nov. is proposed. The type strain is IMMIB L-21^T (=DSM 45234^T=CCUG 56587^T).

The genus *Nocardiosis* was proposed by Meyer (1976) on the basis of chemotaxonomic and morphological characteristics. At the time of writing, the taxon encompasses 28 recognized species and 4 subspecies, which form a distinct clade within the evolutionary radiation occupied by members of the family *Nocardiopsaceae* (Rainey *et al.*, 1996). *Nocardiosis* strains are frequently isolated from saline soils (Yassin *et al.*, 1993a; Li *et al.*, 2004, 2006), but they have also been recovered from an alkaline slag dump (Schippers *et al.*, 2002), indoor environments (Peltola *et al.*, 2001), the atmosphere of a composting facility (Kämpfer *et al.*, 2002) and clinical material (Bernatchez & Lebreux, 1991; Yassin *et al.*, 1997). In the present polyphasic study, an actinomycete isolated from household waste was shown to represent a novel species of the genus *Nocardiosis*.

Strain IMMIB L-21^T was isolated from a household dustbin on Brain Heart Infusion (BHI; BD) agar, glucose-yeast extract-malt extract agar (GYM; medium 65; DSMZ) and Columbia agar (BD) supplemented with 5% sheep blood. The organism was grown on yeast extract-malt extract agar (ISP 2), oatmeal agar (ISP 3) and inorganic salts-starch agar (ISP 4) as described by Shirling & Gottlieb (1966) and examined for pigmentation and colour of aerial and substrate mycelia. Gram and Ziehl–Neelsen stains were applied to hyphae grown on Columbia agar. Growth at different temperatures was determined at 10, 20, 27, 37 and 42 °C and a range of phenotypic characteristics was examined using standard procedures

(Gordon, 1966, 1967; Gordon & Mihm, 1957; Yassin *et al.*, 1995). Tolerance to NaCl (0–16%) was tested in GYM broth. Biomass for chemotaxonomic studies was derived from a 7-day-old BHI broth shake culture incubated at 37 °C, harvested by centrifugation and washed with distilled water. Standard procedures were used to determine the isomeric form of diaminopimelic acid (Becker *et al.*, 1964), whole-cell sugars (Lechevalier, 1968), non-hydroxylated fatty acids (Minnikin *et al.*, 1980; Yassin *et al.*, 2007), polar lipids (Yassin *et al.*, 1993b) and isoprenoid quinones (Collins *et al.*, 1977; Yassin & Hupfer, 2006).

Genomic DNA extraction and PCR-mediated amplification of the 16S rRNA gene sequence were carried out using established procedures (Rainey *et al.*, 1996). The purified PCR products were sequenced using a Taq DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Germany) following the manufacturer's protocol. An ABI 310 Genetic Analyzer (Applied Biosystems) was used for electrophoresis of the sequence-reaction products. The 16S rRNA gene sequences of strain IMMIB L-21^T and those of recognized species of the genus *Nocardiosis*, retrieved from GenBank, were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the respective tool from the ARB package. The resulting alignment was corrected manually and evolutionary trees were inferred using maximum-parsimony (Fitch, 1971), neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) methods. The evolutionary distance matrix for the neighbour-joining method was calculated using the correction of Jukes & Cantor (1969). The

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain IMMIB L-21^T is FM253114.

topologies of the neighbour-joining tree were evaluated using bootstrap analyses (Felsenstein, 1985) based on 500 resamplings.

DNA–DNA relatedness studies were performed between strain IMMIB L-21^T, *Nocardiopsis composta* DSM 44551^T and *Nocardiopsis halophila* DSM 44494^T. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) with consideration of the modifications described by Huß *et al.* (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with an *in situ* temperature probe (Varian).

Strain IMMIB L-21^T grew well on ISP 2–4, producing a pale-yellow to brown–yellow substrate mycelium that carried white aerial hyphae; diffusible pigments were not produced. Melanoid pigments were not produced on ISP 6 or ISP 7. The substrate hyphae fragmented into coccoid-to-rod-shaped elements. The aerial hyphae bore chains of spores. The spores were elongated and had smooth surfaces. The hyphae were Gram-positive and non-acid–alcohol-fast. The organism grew at temperatures up to 37 °C but not at 10 or 42 °C. The range of phenotypic characteristics are listed in Table 1 and the species description.

Chemotaxonomic studies revealed that strain IMMIB L-21^T had a chemical profile consistent with its assignment to the genus *Nocardiopsis*. The strain was characterized by the presence of *meso*-diaminopimelic acid with no characteristic sugars in whole-organism hydrolysates (cell-wall chemotype III according to Lechevalier & Lechevalier, 1970). The respiratory quinones consisted of dihydrogenated, tetrahydrogenated, hexahydrogenated and octahydrogenated menaquinones with 9, 10 and 11 isoprene units [MK-11(H_{2,4,6,8}), MK-10(H_{2,4,6,8}) and MK-9(H_{2,4,6,8})] as the predominant menaquinones. The major phospholipids of strain IMMIB L-21^T were phosphatidylcholine, phosphatidylmethylethanolamine, diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides (phospholipid type III according to Lechevalier *et al.*, 1977). The fatty acid profile was dominated by iso- and anteiso-branched and 10-methyl-branched fatty acids. Traces of straight chain unsaturated fatty acids were detected. The principal components were anteiso-C_{13:0} (0.85 % of total fatty acids), iso-C_{14:0} (3.91 %), C_{14:0} (2.71 %), iso-C_{15:0} (5.34 %), anteiso-C_{15:0} (18.82 %), C_{15:0} (3.05 %), iso-C_{16:0} (9.97 %), C_{16:1ω7c} (1.04 %), C_{18:1ω9c} (3.16 %), C_{16:0} (9.90 %), 10-methyl C_{16:0} (0.59 %), iso-C_{17:0} (2.99 %), anteiso-C_{17:0} (11.16 %), C_{17:1ω8c} (5.90 %), C_{18:0} (5.66 %) and 10-methyl C_{18:0} (4.53 %). All of these chemical properties are consistent with the classification of the isolate in the genus *Nocardiopsis*.

To establish the phylogenetic position of strain IMMIB L-21^T, its 16S rRNA gene sequence was determined in this

Table 1. Differential phenotypic characteristics of strain IMMIB L-21^T, *N. composta* and *N. halophila*

Strains: 1, IMMIB L-21^T (*Nocardiopsis potens* sp. nov.; data from this study); 2, *N. composta* DSM 44551^T (this study); 3, *N. halophila* DSM 44494^T (this study). All strains were positive for hydrolysis of aesculin, casein, hypoxanthine and urea, utilization of L-arabinose, cellobiose, citrate, L-lactate and D-mannitol as carbon sources and utilization of L-alanine, gelatin, L-proline, L-serine and L-ornithine as simultaneous sources of carbon and nitrogen. All strains were negative for hydrolysis of adenine and utilization of *m*-hydroxybenzoate, raffinose, D-sorbitol and D-xylose as carbon sources. +, Positive; –, negative; w, weak utilization after 3 weeks.

Characteristic	1	2	3
Hydrolysis of:			
Elastin	+	–	–
Gelatin	–	+	–
Guanine	+	+	–
Testosterone	+	+	–
L-Tyrosine	+	+	–
Xanthine	–	+	–
Sources of carbon and energy			
Acetate	+	+	–
Adonitol	+	+	–
Adipic acid	+	–	–
iso-Amyl alcohol	+	+	–
2,3-Butanediol	+	–	–
<i>meso</i> -Erythritol	+	+	–
D-Galactose	+	+	–
D-Glucose	+	+	–
D-Gluconate	+	+	w
<i>p</i> -Hydroxybenzoate	–	–	+
<i>myo</i> -Inositol	+	+	–
Lactose	+	+	–
Maltose	+	+	–
Melezitose	+	–	–
1,2-Propanediol	–	+	+
L-Rhamnose	–	+	–
Sucrose	+	+	–
Trehalose	+	+	–
Sources of carbon and nitrogen			
Acetamide	–	+	+

study (1498 nt). Sequence database searches revealed that strain IMMIB L-21^T was most closely related to species of the genus *Nocardiopsis*. A tree constructed using the neighbour-joining method, showing the nearest phylogenetic relatives of strain IMMIB L-21^T, is shown in Fig. 1. The phylogenetic analysis confirmed the placement of strain IMMIB L-21^T within the genus *Nocardiopsis*, with the strain forming a monophyletic clade with *N. composta*. However, the association of strain IMMIB L-21^T with *N. composta* was not statistically significant (bootstrap value of 69 %). Comparative 16S rRNA gene sequence analysis demonstrated that strain IMMIB L-21^T displayed sequence similarity values of less than 98.6 % to the type strains of recognized members of the genus *Nocardiopsis*: highest

Fig. 1. Neighbour-joining tree showing the position of strain IMMIB L-21^T in the genus *Nocardioptis*. Percentages at nodes (>50%) represent levels of bootstrap support based on 500 resamplings. Solid circles indicate that the corresponding nodes were also recovered in maximum-likelihood and maximum-parsimony trees. *Nocardioptis alba* DSM 43377^T was used as the outgroup. Bar, 5.0% sequence divergence.

sequence similarities were shown with *N. composta* DSM 44551^T (98.6 %) and *N. halophila* DSM 44494^T (98.1 %). However, it is clear from the DNA–DNA relatedness study that strain IMMIB L-21^T represents a genomic species separate from *N. composta* DSM 44551^T (DNA–DNA relatedness value 15.2 ± 6.08 %; mean $\pm$ SD) and *N. halophila* DSM 44494^T (6.1 ± 0.63 %). These values are well below the 70.0 % cut-off point recommended for the assignment of strains to the same genomic species (Wayne *et al.*, 1987). It is also apparent that strain IMMIB L-21^T can be distinguished from *N. composta* DSM 44551^T and *N. halophila* DSM 44494^T by a combination of phenotypic properties (Table 1).

It can be concluded from the genotypic and phenotypic data that strain IMMIB L-21^T is phylogenetically distant from its neighbours and distinguishable from them on the basis of its phenotypic properties. Therefore, strain IMMIB L-21^T represents a novel species of the genus *Nocardioptis*, for which the name *Nocardioptis potens* sp. nov. is proposed.

Description of *Nocardioptis potens* sp. nov.

Nocardioptis potens (po'tens. L. part. adj. *potens* powerful, pertaining to the metabolic activities of the organism).

Aerobic, Gram-positive, non-acid–alcohol-fast, non-motile actinomycete. Forms a branched substrate mycelium that fragments into coccoid and rod-shaped elements. Aerial hyphae differentiate into straight to flexuous chains of rod-shaped spores with smooth surfaces. Pale-yellow to brown–yellow substrate mycelium that carries white aerial hyphae is formed on ISP 2–4. Grows at 20–37 °C, but not at 10 or 42 °C. Diffusile pigments are not produced. Melanoid pigment is not produced on ISP media 6 and 7. Hydrolyses aesculin, casein, elastin, guanine, hypoxanthine, testosterone, tyrosine and urea, but not adenine, gelatin or xanthine. Assimilates acetate, adonitol, adipate, iso-amyl alcohol, L-arabinose, 2,3-butanediol, cellobiose, citrate, *meso*-erythritol, D-galactose, D-gluconate, D-glucose, *myo*-inositol, L-lactate, lactose, maltose, D-mannitol, melezitose, sucrose and trehalose as carbon sources but not *m*-hydroxybenzoate, *p*-hydroxybenzoate, 1,2-propanediol, raffinose, L-rhamnose, D-sorbitol or D-xylose. Utilizes L-alanine, arginine, gelatin, proline, ornithine and serine but not acetamide as simultaneous carbon and nitrogen sources. Grows in the presence of 12 % NaCl. Whole-organism hydrolysates contain *meso*-diaminopimelic acid and no characteristic sugars. Major phospholipids are diphosphatidylglycerol, phosphatidylcholine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and phosphatidylmethylethanolamine. Major menaquinones are MK-11(H₈), MK-11(H₆), MK-11(H₄), MK-11(H₂), MK-10(H₈), MK-10(H₆), MK-10(H₄), MK-10(H₂), MK-9(H₈), MK-9(H₆), MK-9(H₄) and MK-9(H₂). Minor amounts of MK-11, MK-8(H₆), MK-8(H₄) and MK-8(H₂) are also present. Major fatty acids are anteiso-C_{15:0}, anteiso-C_{17:0}, iso-C_{16:0} and C_{16:0}. Substantial

amounts of iso-C_{14:0}, iso-C_{15:0}, C_{17:1}ω8, C_{18:1}ω9, C_{18:0} and tuberculostearic acid (10-methyl C_{18:0}) are present.

The type strain is IMMIB L-21^T (=DSM 45234^T=CCUG 56587^T), which was isolated from household waste.

Acknowledgements

We thank Professor Dr Hans-Georg Trüper for nomenclatural advice.

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