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Nocardia alba sp.nov., a Novel Actinomycete Strain Isolated from Soil in China

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Summary

A novel actinomycete strain, designated YIM 30243^T, was isolated from a soil sample in Yunnan Province, China. Based on the results of phenotypic and genotypic characteristics, strain YIM 30243^T should be assigned to a new species of the genus *Nocardia*, for which the name *Nocardia alba* sp. nov. is proposed. The type strain is YIM 30243^T (= CCTCC AA001030^T = DSM 44684^T).

Key words: *Nocardia alba* sp.nov. – Polyphasic taxonomy – 16S rDNA

Introduction

The application of chemotaxonomic and molecular systematic methods promoted a radical reappraisal of the genus *Nocardia* [5, 19, 32]. The improved classification provides a sound framework for the recognition of additional species [36]. Members of the genus form extensively branched, substrate hyphae which fragment into rod-shaped, non-motile elements; aerial hyphae are usually formed but are sometimes only visible microscopically [8, 9, 10]. Nocardiae are also characterized by a number of chemical markers, including the presence of *meso*-DAP, arabinose and galactose, mycolic acids with 40–60 carbon atoms and DNA G+C content of 64–72% [4, 8].

In this work, we present the polyphasic taxonomic characterization of strain YIM 30243^T, Genotypic and phenotypic data showed that the strain YIM 30243^T should be recognized as a new species for which the name *Nocardia alba* sp. nov. is proposed.

Methods

Micro-organisms and culture conditions

Strain YIM 30243^T was isolated, using the dilution plating method, from a soil sample in HeKou, Yunnan Province, the west of China. The medium used for selective isolation was HV agar [11] and incubated at 28 °C for about 2 weeks. The strain was maintained on ISP2 agar or ISP5 agar slants at 4 °C and as glycerol suspensions (20%,v/v) at –20 °C. Biomass for chemical and molecular systematic studies was obtained by growing in shake flasks (about 150 rpm.) of ISP2 medium broth at 28 °C for 1 week.

Phenotypic characteristics

Cultural characteristics were determined after 4 weeks at 28 °C by methods used in the International *Streptomyces* Project (ISP) [28]. Morphological properties were examined by light microscopy (Olympus microscope BH-2) and scanning electron microscopy with a JEOL model JSM5600LV. Media and procedures used for determination of physiological features and carbon source utilization were those described by Shirling, Gottlieb [28] and Locci [21]. Color determination was done with color chips from the ISCC-NBS COLOR CHARTS Standard Samples No 2106 [13].

Chemotaxonomy

The amino acid and sugar analysis of whole cell hydrolysates followed procedures described by Stanek and Roberts [29]. Polar lipids were extracted, examined by two dimensional thin layer chromatograph and identified using published procedures [25]. Menaquinones were isolated using the methods of Minnikin et al. [25] and separated by HPLC [17, 18]. Fatty acid methyl esters and mycolic acid trimethylsilyl esters were prepared and analyzed as described previously [16] using the standard Microbial Identification System (MIDI inc.) for automated GC analyses [27].

DNA G+C content determination

DNA for the base composition and renaturation studies was prepared following the method of Marmur [23]. The G+C content was determined using the thermal denaturation method of Marmur, Doty [24].

The GenBank accession number for the sequence reported in this paper is AY222321.

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Extraction of Genomic DNA and amplification of 16S rDNA

Extraction of Genomic DNA and amplification of 16S rDNA were done as described by Xu et al. [34]. Multiple alignments with sequences of a broad selection of *Actinobacteria* and calculations of levels of sequences similarity were carried out using CLUSTAL X [31]. A phylogenetic tree was reconstructed using neighbor-joining method of Saitou and Nei [26] from K_{nuc} values [14, 15]. The topology of the phylogenetic tree was evaluated by bootstrap resampling method of Felsenstein [3] with 1,000 replicates.

Nucleotide sequence accession numbers

The 16S rDNA sequence of strain YIM 30243^T determined in this study has been deposited in GenBank under the accession number AY222321. The accession numbers of the reference strains, which are closely related to strain YIM 30243^T, are indicated in Fig. 2.

Results

Morphological observations

Morphological observation of a 28-day-old culture of strain YIM 30243^T grown on yeast-malt ext agar (ISP medium 2) revealed it had the typical characteristics of genus *Nocardia* (Fig. 1). Strain YIM 30243^T formed extensively branched substrate hyphae which fragment into rod-shaped, non-motile elements; Aerial mycelium consisting of hyphae that fragmented into short to elongated rod-like elements.

Cultural characteristics

As shown in Table 1. Strain YIM30243^T moderately developed yellow white to pink white for aerial mycelium and substrate mycelium on most media tested. No diffusible pigments were produced. It developed aerial hyphae on all media tested, especially yeast-malt extract agar (ISP medium 2) and glycerol-asparagine (ISP medium 5).

Physiological and biochemical characteristics

Some physiological and biochemical properties of strain YIM 30243^T are shown in Table 2. Strain YIM 30243^T utilized glucose, mannose, mannitol, inositol, xylose, sucrose, maltose, lactose, rhamnose and sodium acetate, but not arabinose, galactose, raffinose, sorbitol and sodium citrate. It was positive for gelation liquefaction, starch hydrolysis and nitrate reduction, but negative for milk coagulation and peptonization, growth in cellulose, H_2S and melanin production.

Chemotaxonomic characteristics

The cell walls of strain YIM 30243^T contained *meso*-diaminopimelic acid. Whole-cell hydrolysates contained mainly galactose and arabinose. The predominant menaquinone was MK-8(H_{4cycl}). The polar lipid extract contained diphosphatidylglycerol, phosphatidyl-ethanolamine, glycolipid, phosphatidylinositol and phosphatidylinositol mannosides (phospholipid type II *sensu* 20). Cellular fatty acids and Mycolic acids are indicated in the species description. The G+C content of the genomic DNA from strain YIM 30243^T was 74 mol%.

Phylogenetic analysis

Almost complete 16S rDNA sequence data of strain YIM 30243^T (1446 bp) was determined. BLAST search results of strain YIM 30243^T came from non-redundant GenBank+EMBL+DDBJ+PDB, and sequences have been chosen as reference sequences in which unidentified and unpublished sequences were not included. Comparison of this sequence with those of related organisms showed clearly that it contained all of the signature nucleotides designated for the family *Nocardiaceae* [30]. The phylogenetic tree (Fig. 2) from representative strains of the related species indicated that strain YIM 30243^T should be in genus *Nocardia* and form a separate lineage together with the other four type strains including *N. cummidelens*, *N. salmonicida, N. fluminea* and *N. ignorata*.

Discussion

The values for 16S rRNA gene sequence similarity to members of the genus *Nocardia* (95.75–97.69%) support the addition of the strain to this genus. This assignment is also supported by the fact that the 16S rDNA sequence contains the signature nucleotide characteristic for the genus *Nocardia* [1]. It is evident from the 16S rDNA trees (Fig. 1) that the isolate is associated with *N. cummide-*

Table 1. Cultural characteristics of strain YIM 30243^T.

Medium		Growth	Aerial mycelium	Substrate mycelium
Yeast extract-malt extract	(ISP* medium 2)	Good	Light gray brown	Yellow brown
Oatmeal agar	(ISP* medium 3)	Good	Pink white	Yellow white
Inorganic salt-starch agar	(ISP* medium 4)	Good	Pink white	Pink white
Glycerol-asparagine	(ISP* medium 5)	Good	Light gray brown	Light yellow brown
Czapek's agar		Good	Pink gray	Pink white
Nutrient agar		Moderate	Yellow white	Light yellow

Note: Diffusible pigments were not produced on any of the media listed.
Colors taken form ISCC-NBS COLOR CHARTS (Standard Samples No 2106) [13].
* ISP, International Streptomyces Project [28].

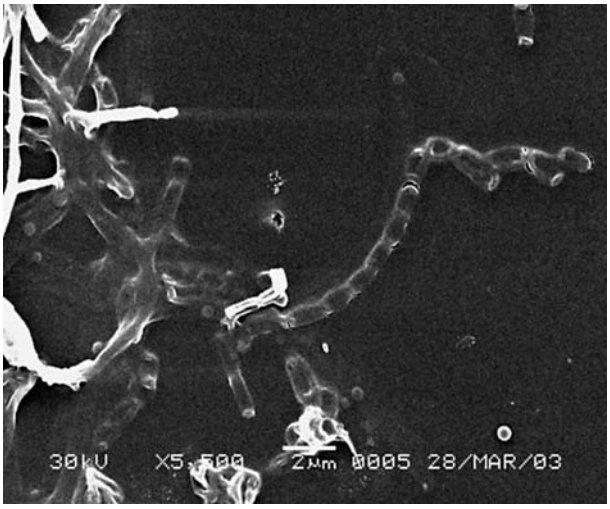

Fig. 1. Scanning electron micrograph of strain YIM 30243^T, grown on yeast-malt extract agar (ISP 2 medium) for 28 days at 28 °C. Bar, 2 μm.

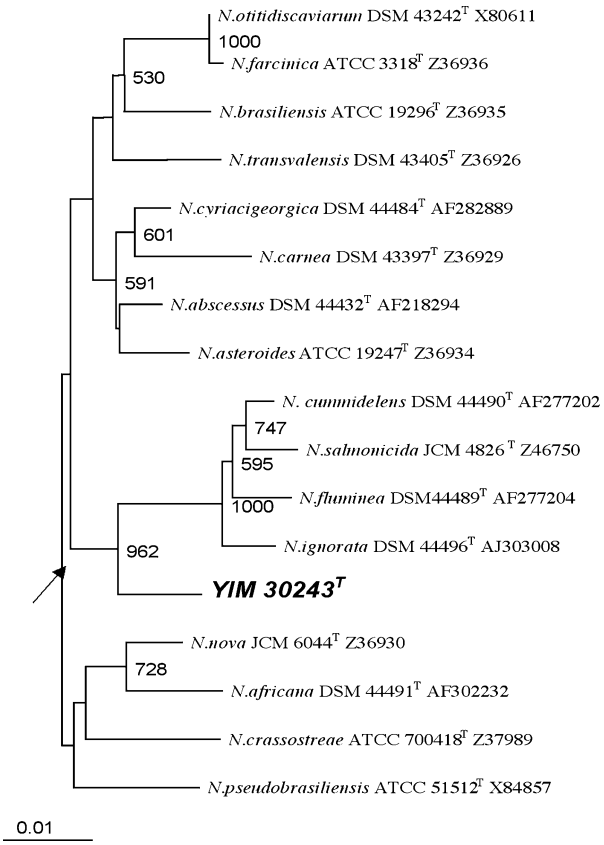

Fig. 2. Phylogenetic dendrogram obtained by distance matrix analysis of 16S rDNA sequences, showing the position of strain YIM30243^T among phylogenetic neighbors. Numbers on branch nodes are bootstrap values (1000 resamplings). The sequence of *Streptosporangium roseum* DSM 43021^T(X89947) was used as root. Bar, 1% sequence divergence.

lens, *N. salmonicida, N. fluminea* and *N. ignorata*. It shares higher 16S rDNA similarity (97.69%, 97.12%, 97.33%, 97.25%, respectively) with the latter four type strains of genus *Nocardia*. Higher similarity values have been recorded between several validly described *Nocardia* species, for instance, between *N. brevicatena* and *N. paucivorans*(99.6%) [35] and *N. carnea* and *N. flavorosea* (99.2%) [2]; the DNA-DNA relatedness values shown between these respective sets of type strains were found to be well below the 70% cut-of point recommended for the circumscription of bacterial genomic species [33].

The results of the morphological, physiological, biochemical and chemical characteristics of strain YIM 30243^T are consistent with its assignment to the genus *Nocardia* [6, 7]. The important chemotaxonomic markers for members of genus *Nocardia* are mycolic acids and fatty acids compositions. While strain YIM 30243^T synthesized mycolic acids ranging from C48 to C56, C52 being principal mycolic acid (about 40%). C50 and C54 occurred in equal amounts (25%) whereas C48 and C56 were minor mycolic acids each about 5%. the predominant cellular fatty acids of strain YIM 30243^T were $C_{16:0}$ (33.9%), cis-9-$C_{16:1}$ (19.2%), cis-9-$C_{18:1}$ (17.6%), 10-Methyl-$C_{18:0}$ (15.0%). Additionally, the DNA G+C content was 74 mol%, which is the highest value reported (the other reported *Nocardia* species are between 64–72 mol%) [4, 8] in genus *Nocardia*. These phenotypic properties distinguishes strain YIM 30243^T from representatives of all validly described species of *Nocardia*, including the four type strains *N. cummidelens*, *N. salmonicida, N. fluminea* and *N. ignorata* in the same branch (Table 2).

Therefore, based on the results of its phenotypic and genotypic data, we propose that strain YIM 30243^T should be classified as a new member of genus *Nocardia*, *Nocardia alba* sp. nov.

Description of *Nocardia alba* sp. nov.

Nocardia alba ('al.ba. L. adj. *alba*, white color). Aerobic, Gram-positive, catalase-positive, the cell of the organism were non-motile. Aerial mycelium and substrate hyphae are extensively branched and fragmented irregularly into rod-shaped, non-motile elements. It utilizes glucose, mannose, mannitol, inositol, xylose, sucrose, maltose, lactose, rhamnose and sodium acetate. but not arabinose, galactose, raffinose, sorbitol and sodium citrate. It is positive for gelation liquefaction, starch hydrolysis and nitrate reduction, but negative for milk coagulation and peptonization, growth in cellulose, H_2S and melanin production. The cell wall of strain YIM 30243^T contains *meso*- diaminopimelic acid. Whole-cell sugars are galactose and arabinose. The principal menaquinone is MK-8(H_{4cycl}). The phospholipids are diphosphatidylglycerol, phosphatidyl- ethanolamine, glycolipid, phosphatidylinositol and phosphatidylinositol mannosides. The predominant cellular fatty acids are $C_{16:0}$ (33.9%), cis-9-$C_{16:1}$ (19.2%), cis-9-$C_{18:1}$ (17.6%), 10-Methyl-$C_{18:0}$ (15.0%).

Table 2. Phenotypic characteristics that distinguish strain YIM 30243^T from the other four related type strains of genus *Nocardia*.

Character	*N. alba* YIM 30243^T	*N. cummidelens* DSM 44490^T	*N. salmonicida* JCM 4826^T	*N. fluminea* DSM 44489^T	*N. ignorata* DSM 44496^T
Carbon utilization					
Glucose	+	+	+	+	+
Mannitol	+	–	+	–	+
Inositol	+	–	–	+	–
Arabinose	–	ND	–	ND	–
Maltose	+	ND	–	ND	+
Galactose	–	ND	+	ND	–
Raffinose	–	ND	+	ND	–
Rhamnose	+	–	–	+	–
Sorbitol	–	–	–	–	W
Sodium acetate	+	+	ND	–	ND
Sodium citrate	–	–	ND	+	ND
Growth at 45 °C	–	–	–	–	+
G+C mol%	72	ND	67	ND	68
Mycolic acids (carbon atoms)	48–54	ND	46–60	ND	46–57
Fatty acids (>10%)	$C_{16:0}$ (33.9%); cis-9-$C_{16:1}$ (19.2%); cis-9-$C_{18:1}$ (17.6%), 10-Methyl-$C_{18:0}$ (15.0%)	ND	$C_{16:0}$ 32%; $C_{16:1}$ 14%; $C_{18:0}$ 16%; 10-Methyl-$C_{18:0}$ 25%	ND	$C_{16:0}$ 67.14%; $C_{18:0}$ 25.3%

Data were taken from this and previous studies (12, 22, 37). +, Positive; –, negative; w, utilized weakly; d, doubtful; ND, not determined. All taxa listed utilized glucose as sole sources of carbon for growth.

The mycolic acids ranging from C48 to C56, C52 is principal mycolic acid (about 40%). C50 and C54 are in equal amounts (25%), whereas C48 and C56 are minor, each about 5%. The DNA G+C content is 74 mol%. Its optimum growth temperature and pH are at 28 °C and 7.0, respectively. It was isolated from soil in Yunnan Province, the west of China. The type strain is strain YIM 30243^T (= CCTCC AA001030^T = DSM 44684^T).

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