Nocardia vinacea sp. nov.

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This paper reports taxonomical studies on a soil isolate, the strain MK703-102F1, which produces a new antimycobacterial antibiotic tubelactomicin. We propose that this strain should belong to a new species of the genus *Nocardia* with the new name *Nocardia vinacea* sp. nov., the type strain being MK703-102F1 (= JCM 10988 = IFO 16497). This new species was characterized by *meso*-diaminopimelic acid as a cell wall diamino acid, arabinose and galactose as whole-cell sugars, *N*-glycolated muramic acids in cell walls, type PII phospholipids, MK-8(H₄) menaquinone, fatty acid components consisting of hexadecanoic (16:0), octadecenoic (18:1), 10-methyloctadecanoic (10Me-18:0) and hexadecenoic (16:1) acids, mycolic acids with 48–56 carbons, some physiological characteristics, 65 mol% G+C in DNA, and base sequence of 16S ribosomal RNA gene (16S rDNA).

INTRODUCTION

In a course of screening for new anti-microbial substances from microorganism origin, we found an actinomycete strain MK703-102F1 to produce tubelactomicin A^{1,2}), a new 16-membered lactone antibiotic, showing strong and specific activity against Rapid Growers *Mycobacterium*¹). We briefly described some taxonomic characteristics of MK703-102F1¹). Further taxonomic studies on this strain have been continued in detail and we propose in the present paper the name *Nocardia vinacea* for this strain as a new species.

MATERIALS AND METHODS

Organism: Strain MK703-102F1 was isolated from a soil sample collected at Suwa-shi, Nagano, Japan. Because of some genotypic and/or phenotypic similarities, this strain was taxonomically compared with *Nocardia asteroides* IMC A-0221 (JCM 3384^T)(T=Type strain), *N. abscessus* IMC A-0223 (JCM 10984^T), *N. brasiliensis* IMC A-0198 (JCM 3374^T), *N. carnea* IMC A-0212 (JCM 3375^T), *N. nova* IMC A-0216 (JCM 6044^T), *N. otitidiscaviarum* IMC A-0213 (JCM 3377^T), *N. paucivorans* IMC A-0222 (JCM 10919^T), *N. pseudobrasiliensis* IMC A-0215 (JCM 9894^T), *N. salmonicida* IMC A-0181 (ISP 5472^T), *N. transvalensis* IMC A-0214 (JCM 9099^T) and *N. vaccinii* IMC A-0217 (JCM 3395^T).

Morphological characterization: Morphological observations under a light microscope and a scanning electron microscope (model S-570, Hitachi) were made on cultures grown on sucrose-nitrate agar and glucose-asparagine agar, at 27°C for 4 to 21 days. For scanning electron microscope inspections, the cultures were fixed in 2% osmium tetroxide vapor *in situ* at 4°C for 18 hours, frozen in liquid nitrogen, and then freeze-dried. Each specimen was coated with gold using an ion sputter instrument (model SC-701 quick coater, Sanyu Denshi). The motility

of spores was observed as reported³).

Cultural and physiological characterization: Experiments were conducted as reported^{4–7}). Permissive temperatures for growth were determined on glucose-asparagine agar.

Chemotaxonomic characterization: Cells used for chemotaxonomic analysis were obtained upon incubating the organism at 27°C for 4 days in yeast extract-glucose broth (1.0% yeast extract, 1.0% glucose, pH 7.2) on a rotary shaker. Whole cell hydrolysates were analyzed for diaminopimelic acid isomers using thin layer chromatography (TLC)⁸). Whole-cell sugars were prepared as reported⁹) and analyzed using TLC¹⁰). The cell wall acyl type was determined as reported¹¹). Phospholipids were analyzed using TLC¹²). Mycolic acids were prepared as reported¹³) and analyzed using a mass spectrometer (model M-80H, Hitachi). Menaquinones were extracted as reported¹⁴), and analyzed in an LC-MS (model M-1200H, Hitachi) with a CAPCELL PAK C₁₈ column (150 mm by 4.6 mm, Shiseido) using methanol-isopropanol (2:1, v/v) as the mobile phase. Methylesters of cellular fatty acids were prepared as reported¹⁵) and analyzed using a gas chromatography set (model GC-17A, Shimadzu) which consisted of a Megabore DB-1 column (15 m by 0.35 mm, film 1.5 µm, J & W Scientific), a flame ionization detector, and a Shimadzu Chromatopak C-R6A.

DNA analysis: A total DNA sample was prepared¹⁶) and the G+C content was determined using HPLC, as reported¹⁷). Genome homologies were examined by fluorometric DNA-DNA hybridization in microdilution wells¹⁸).

The 16S rDNA was amplified by PCR using genomic DNA of MK703-102F1 and sequenced¹⁹).

The sequences of the 16S rDNA from the following organisms were informed from EMBL, GenBank, and DDBJ (DNA Data Base of Japan, Mishima, Japan) for comparison: *Nocardia asteroides* ATCC 19247^T (Z36934), *N. abscessus* IMMIB D-1592^T (AF218292), *N. brasiliensis* ATCC 19296^T (Z36935), *N. brevicatena* DSM 43024^T

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(Z36928), *N. carnea* DSM 43397^T (Z36929), *N. crassostreae* NB4H^T (Z37989), *N. farcinica* ATCC 3318^T (Z36936), *N. flavorozea* JCM 3332^T (Z46754), *N. nova* JCM 6044^T (Z36930), *N. otitidiscaviarum* DSM43242^T (X80611), *N. paucivorans* IMMIB D-1632^T (AF179865), *N. pseudobrasiliensis* ATCC 51512^T (X84857), *N. salmonicida* JCM 4826^T (Z46750), *N. seriolae* JCM 3360^T (Z36925), *N. transvalensis* DSM 43405^T (Z36926), *N. uniformis* JCM 3224^T (Z46752), *N. vaccinii* DSM 43285^T (Z36927), *N. veterana* DSM 44445^T (AF278572) and *Streptomyces ambofaciens* ATCC 23877^T (M27245). The alignment used in the phylogenetic analyses comprised 1340 nt positions between positions 41 and 1408 (*Escherichia coli* numbering system²⁰). Phylogenetic tree was constructed by the neighbor-joining (NJ) method²¹ using Clustal W software²².

The sequence of the newly determined 16S rDNA is available from DDBJ under the accession number: *Nocardia vinacea* MK703-102F1 (= JCM 10988 = IFO 16497), AB024312.

RESULTS AND DISCUSSION

Morphological characteristics: Substrate mycelia of MK703-102F1 were well branched and fragmented. Aerial mycelia were straight, flexuous or spirals (Fig. 1), and were fragmented into cylindrical and ellipsoidal spore-like elements (0.4 to 0.6 by 0.8 to 1.8 μ m). The surface of spore-like elements was smooth. None of synnemata, sclerotia, sporangia and motile spores was observed.

Cultural and physiological characteristics: The cultural characteristics of MK703-102F1 are shown in Table 1. Aerial mycelia were white to brownish white, and substrate mycelia were colorless to pale yellow to pale brown. Pale reddish purple soluble pigments were produced in glucose-asparagine agar and glycerol-asparagine agar (ISP medium No.5). The physiological characteristics are shown in Table 2. Permissive temperatures for growth

ranged from 20°C to 37°C, with the optimal temperature at 30°C. Additional data on physiological characteristics are given in the section of Description of *Nocardia vinacea* sp. nov..

Chemotaxonomic characteristics: Whole-cell hydrolysates of MK703-102F1 contained *meso*-diaminopimelic acid as a cell wall diamino acid, arabinose and galactose as whole-cell sugars. *N*-acyl type of muramic acid in cell walls was glycolyl type. Phospholipid pattern was type PII; phosphatidylethanolamine was present but none of phosphatidylcholine or unknown glucosamine-containing phospholipids was found. Mycolic acids with 48–56 even number carbons were present. About 80% menaquinones were MK-8(H₄). Fatty acids consisted of hexadecanoic acid (16:0) at 48%, octadecenoic acid (18:1) at 21%, 10-methyloctadecanoic acid (10Me-18:0) at 13%, hexadecenoic acid (16:1) at 10%, and other minor components. The G+C content of DNA was 65 mol%. These chemotaxonomic characteristics suggested that MK703-102F1 belonged to genus *Nocardia*.

Phylogenetic analysis based on DNA sequence homology and classification of MK703-102F1: The partial 16S rDNA sequence (1340 nt) of MK703-102F1 was compared with the corresponding sequences of 18 species of genus *Nocardia* and, as a root, that of *Streptomyces ambofaciens*. MK703-102F1 showed similarity values of 96.0 to 97.8% with the 18 species of *Nocardia*, while only 88% with *S. ambofaciens*. The phylogenetic tree by neighbor-joining method (Fig. 2) showed that MK703-102F1 positioned in a cluster with *N. nova* (97.8%), *N. vaccinii* (97.6%) and *N. veterana* (96.7%).

Genome DNA hybridization was performed between MK703-102F1 and the *Nocardia* species that had shown 97.0% or higher values of similarity in the 16S rDNA sequence and the results are shown in Table 2. MK703-102F1 did not show any significant homology with others including *N. nova* and *N. vaccinii* that formed the same cluster with MK703-102F1 in Fig. 2.

To confirm that MK703-102F1 is a new species of *Nocardia*, some physiological characteristics were compared between MK703-102F1 and the 18 species of *Nocardia*. None of the 18 species completely matched MK703-102F1 in this test (Table 3). *N. nova* and *N. vaccinii*, that located in the same cluster with MK703-102F1 (Fig. 2), differed from MK703-102F1 in the metabolism of hypoxanthine, D-sorbitol, D-mannitol or L-rhamnose. In addition, a pale reddish purple soluble pigment of MK703-102F1 on glucose-asparagine agar was not found in any species compared.

Judging from the genotypic and phenotypic data described above, we have concluded that MK703-102F1 is a new species

Fig. 1. Scanning electron micrographs of strain MK703-102F1 grown on sucrose-nitrate agar at 27°C for 6 days. Bar = 1 μ m.

Table 1. Cultural characteristics of strain MK703-102F1.

Medium	Growth	Aerial mycelium	Soluble pigment
Sucrose-nitrate agar	Colorless	Thin, white	None
Glucose-asparagine agar	Pale orange [3 gc, Lt Tan]	White	Shade of pale red dish purple
Yeast extract-malt extract agar (ISP No. 2)	Pale yellow [2 ec, Sand] ~pale brown [3 ie, Camel]	Brownish white [2 ba, Pearl]	None
Oatmeal agar (ISP No. 3)	Colorless	Thin, white	None
Inorganic salts-starch agar (ISP No. 4)	Colorless	Thin, white	None
Glycerol-asparagine agar (ISP No. 5)	Pale yellow [2 ec, Sand] ~pale brown [3 ie, Camel]	Brownish white [2 ba, Pearl]	Shade of pale reddish purple
Tyrosine agar (ISP No. 7)	Pale yellow [2 ec, Sand] ~pale brown [3 ie, Camel]	Brownish white [2 ba, Pearl]	None

Observations with the cultures grown at 27°C for 21 days.

Color names and numbers follow Color Harmony Manual, Container Corporation of America.²³⁾

Table 2. Total DNA homologies between various species of *Nocardia*, including strain MK703-102F1.

Plate-bound DNA	%Hybridization with photobiotin labeled DNA							
	Strain MK703- 102F1	<i>N. asteroides</i> JCM 3384 ^T	<i>N. nova</i> JCM 6044 ^T	<i>N. pseudo- brasiliensis</i> JCM 9894 ^T	<i>N. transvalensis</i> JCM 9099 ^T	<i>N. vaccinii</i> JCM 3395 ^T	<i>N. abscessus</i> JCM 10984 ^T	<i>N. paucivorans</i> JCM 10919 ^T
Strain MK703-102F1	100	10	9	9	5	4	4	4
<i>N. asteroides</i> JCM 3384 ^T	10	100	14	12	11	8	8	7
<i>N. nova</i> JCM 6044 ^T	7	10	100	13	6	8	6	5
<i>N. pseudobrasiliensis</i> JCM 9894 ^T	7	11	16	100	12	8	3	6
<i>N. transvalensis</i> JCM 9099 ^T	7	12	23	17	100	11	4	6
<i>N. vaccinii</i> JCM 3395 ^T	6	9	16	13	9	100	2	4
<i>N. abscessus</i> JCM 10984 ^T	7	8	9	7	6	5	100	5
<i>N. paucivorans</i> JCM 10919 ^T	5	9	11	8	6	5	4	100

Fig. 2. Unrooted neighbor-joining tree based on 1340 unambiguously aligned nucleotide positions in 16S rRNA gene. *Streptomyces ambofaciens* ATCC 23877^T (M27245) was used as the root organism. Bar, 0.01 K_{nuc} in nucleotide sequences.

Table 3. Physiological characteristics of MK703-102F1 and other species of genus *Nocardia*.

	Strain MK703-102F1	<i>N. asteroides</i> ⁷⁾	<i>N. abscessus</i> ²⁴⁾	<i>N. brasiliensis</i> JCM 3374 ^T	<i>N. brevicatena</i> ⁷⁾	<i>N. carnea</i> JCM 3375 ^T	<i>N. crassostreae</i> ²⁵⁾	<i>N. farcinica</i> ⁷⁾	<i>N. flavorosea</i> ²⁶⁾	<i>N. nova</i> JCM 6044 ^T	<i>N. otitidiscaviarum</i> JCM 3377 ^T	<i>N. paucivormis</i> ²⁷⁾	<i>N. pseudobrasiliensis</i> JCM 9894 ^T	<i>N. salmonicida</i> ISP 5472 ^T	<i>N. seriolae</i> ^{7,28)}	<i>N. transvalensis</i> JCM 9099 ^T	<i>N. uniformis</i> ²⁹⁾	<i>N. vaccinii</i> JCM 3395 ^T	<i>N. veterana</i> ³⁰⁾
Biochemical tests																			
Aesculin hydrolysis	d	+	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	d*	ND
Nitrate reductase	+	+	ND	+	-	+	ND	+	-	+	+	ND	d*	-*	-	+	+	+	ND
Urea hydrolysis	+	+	+	+	-	-	-	+	-	+	+	+	+	+	-	+	+	-*	ND
Decomposition of																			
Adenine	-	-	-	-	-	-	ND	-	-	-	-	-	+	-	-	+	-	-	ND
Casein	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	ND
Elastin	-	-	-	+	-	-	ND	-	-	-	-	-	+	-	-	-	+	-	ND
Hypoxanthine	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	+	+	-	ND
Testosterone	+	+	+	+	+	+	ND	+	+	+	+	-	d*	+	-	+	+	+	ND
Tyrosine	-	-	-	+	-	-	-	-	-	d*	+	-	+	+	-	+	+	-	ND
Uric acid	+	-	ND	+	ND	+	ND	-	+	+	+	ND	+	+	-	+	+	+	ND
Xanthine	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	ND
Utilization of																			
D-Mannitol (1%)	+	-	-	+	-	+	ND	-	-	-	+	-	d*	-*	-	+	-	+	ND
L-Rhamnose (1%)	-	-	+	-	+	-	ND	+	-	-	-	-	-	-	-	-*	-	+	+
D-Sorbitol (1%)	+	-	-	-	-	-*	ND	-	-	-	-	-	-*	-*	-	+	-	-	ND
Sodium citrate (0.1%)	d	+	+	d	-	-	-	-	-	-	-	-	-*	d*	+	d*	-	-	-
Growth at																			
30°C	+	+	ND	+	+	+	+	+	+	+	+	ND	+	+	+	+	+	+	ND
37°C	+	+	ND	+	+	+	-	+	+	+	+	ND	+	-	-	+	+	+	ND
45°C	-	-	ND	-*	-	-	-	+	+	-	-	ND	-	-	-	-	-	-	ND

Abbreviations: ND, no data available; v, variable; d, doubtful.

*: Different from reference description.

of genus *Nocardia*. We propose, therefore, the name *Nocardia vinacea* for MK703-102F1, as a new species in the genus *Nocardia*.

Description of *Nocardia vinacea* sp. nov. : *Nocardia vinacea* (*vi.na'ce.a* L. adj. derived from L. neut. n. *vinum* wine; M.L. adj. *vinacea* referring to the pale reddish purple soluble pigment).

The substrate mycelia are fragmented. The aerial mycelia are straight, flexuous or spiral, and are fragmented into cylindrical and ellipsoidal spore-like elements. The surface is smooth. The substrate mycelia are colorless to pale yellow to pale brown. The aerial mycelia are white to brownish white. The soluble pigments are pale reddish purple or not produced depending on culture conditions. Melanoid pigments are not produced. L-Arabinose, D-glucose, D-fructose, D-mannitol and D-sorbitol are utilized, but not D-xylose, sucrose, *myo*-inositol, rhamnose or raffinose. Hypoxanthine, testosterone, urea and uric acid are hydrolyzed, but not adenine, casein, elastin, starch, tyrosine or xanthine. Acid is produced from adonitol, L-arabi-

nose, D-glucose, glycerol, *myo*-inositol and D-sorbitol; no acid is produced from cellobiose, dextrin, *meso*-erythritol, lactose, melibiose, raffinose, L-rhamnose, salicin, sucrose, D-xylose or methyl- α -glucoside. DL-Tartrate are assimilated, but not benzoate or oxalate. The strain grows in the presence of 4% NaCl. The temperature range for growth is 20 to 37°C. Nitrate is reduced to nitrite. Phosphatase is produced. The G+C content of DNA is 65 mol%. It is isolated from soil. The type strain of *Nocardia vinacea* is the strain MK703-102F1 (= JCM 10302 = IFO 16184). Data on the analysis of DNA sequence homology are included.

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