Jpn. J. Med. Mycol.
Vol. 46, 21－26, 2005
ISSN 0916－4804

Original Article

Nocardia anaemiae sp. nov. Isolated from an Immunocompromised Patient and the First Isolation Report of *Nocardia vinacea* from Humans

Akiko Kageyama, Katsukiyo Yazawa, Kazuko Nishimura, Yuzuru Mikami
Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University,
1-8-1 Inohana, Chuo-ku, Chiba 260-8673, Japan
〔Received: 8, June 2004. Accepted: 25, October 2004〕

Abstract

Two actinomycete strains that were isolated from patients in Japan were assigned provisionally to the genus *Nocardia* based on their morphological characteristics. The two isolates were further studied to determine their specific taxonomic status. Detailed chemotaxonomic characterization and 16S rDNA sequence data for the strains showed that they are most similar to *Nocardia vinacea*. DNA-DNA hybridization experiments indicated that strain IFM 0344 should be identified as *N. vinacea*, and that strain IFM 0323^T is classifiable as a new species. This report describes the first isolation of *N. vinacea* from clinical samples. A new species of the genus *Nocardia* is proposed based on their phenotypic and phylogenetic characteristics: *Nocardia anaemiae* for IFM 0323^T (=NBRC 100462^T=JCM 12396^T=DSM 44821^T).

Key words: *Nocardia vinacea*, first isolate in Japan, *Nocardia anaemiae*

Introduction

Most *Nocardia* species can cause human infections that are difficult to diagnose because of their non-specific clinical and histological manifestations[1-3]. Definitive diagnosis depends on the isolation and identification of a causative *Nocardia* species[3, 4]. Nocardiosis has been considered rare, however, recently the incidence of infection is increasing[5].

Classification of *Nocardia* species using a combination of genotypic and phenotypic methods has led to the establishment of more than 20 new species[6, 7]. *Nocardia vinacea* was also recently described by Kinoshita *et al.*[8]. The type strain was isolated from soil. It was found to produce a 16-membered lactone group antibiotic: tubelatomicin A[8].

During taxonomic studies of *Nocardia asteroides*-like clinical isolated strains, we found that two clinical isolates, strains IFM 0344 and IFM 0323^T, have phenotypic characteristics that are similar to *N. vinacea*. DNA-DNA hybridization showed that strain IFM 0344 belongs to *N. vinacea*. This report describes the first case of an infection caused by this species. DNA-DNA hybridization also showed that strain IFM 0323^T can be delineated from *N. vinacea*. Hence, we propose strain IFM 0323^T as a new species: *Nocardia anaemiae* sp. nov.

Address for correspondence: Yuzuru Mikami
1-8-1 Inohana, Chuo-ku, Chiba 260-8673, Japan
Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba university

Materials and Methods

Bacterial strain and media

Strains IFM 0323^T, IFM 0344, and *N. vinacea* IFM 10175^T were cultured at 27°C for seven days on a Muller Hinton II (MH II) agar slant supplemented with 1% glucose and 1% glycerol. Strain IFM 0323^T was isolated in 1989 from a 73-year-old Japanese male patient with a history of autoimmune hemolytic anemia (AIHA) and steroid therapy. Strain IFM 0344 was isolated in 1990 from a Japanese patient without a detailed history. For extraction of DNA and its sequencing, the bacterial strains were cultured at 32°C for four days in brain heart infusion (BHI; Difco Laboratories) broth.

Biochemical and chemotaxonomic characteristics

Respective decompositions of adenine, casein, hypoxanthine, tyrosine, urea, and xanthine were examined as in Gordon *et al.*[1]. Acid production

from carbohydrates, utilization of organic acids, and growth temperature were determined by the modified method of Poonwan *et al.*[9].

Whole-cell hydrolysates were analyzed for the diaminopimelic acid (DAP) isomers[10] and for cell wall reducing sugars[11, 12] using thin layer chromatography (TLC). Mycolic acids were analyzed in the manner reported in Minnikin *et al.*[13]. Menaquinones were extracted from freeze-dried biomass (500 mg) and analyzed as described by Chun and Goodfellow[14].

Preparation of genomic DNA samples

A 1-m*l* volume of cultured broth was centrifuged at 12,000 rpm for 10 min. The pellet was resuspended in 200 μl of TE buffer, 250 μl of GPT reagent (6M guanidine thiocyanate dissolved in 50 mM Tris pH 8.3) and 450 μl Tris buffered phenol (pH 8.0). The tube was placed in a boiling water bath for 15 min and extracted with 250 μl of chloroform-isoamyl alcohol (24 : 1 by volume). After 10-min centrifugation at 12,000 rpm, the aqueous phase (ca. 500 μl) was transferred to a fresh tube. The aqueous phase was mixed with 500 μl of 100% isopropanol and 50 μl of 3M sodium acetate. Then it was microcentrifuged at 12,000 rpm for 15 min at 4°C before the supernatant was decanted. Traces of GPT reagent were removed by the addition of 500 μl of ice-cold 70% ethanol to the nucleic acid pellet. That mixture was again centrifuged at 12,000 rpm for 5 min at 4°C. The ethanol was removed. The pellet was dried under a vacuum for 20 min and finally resuspended in 50 μl of TE buffer.

16S rDNA sequencing

16S rDNA was amplified by polymerase chain reaction (PCR) and sequenced using six prokaryotic 16S rDNA universal primers[15, 16]. PCR was performed by a DNA thermal cycler (TaKaRa Shuzo Co., Ltd., Japan) using 35 cycles, each consisting of denaturation at 94°C for 60 s, primer annealing at 60°C for 60 s, and primer extension at 72°C for 120 s. The PCR products were purified using a Centri-Sep Column (Princeton Separations, Inc.). DNA sequences were determined by an automatic sequence analyzer (ABI PRISM™ 3100; PE Applied Biosystems, U.S.A.) using a dye terminator cycle sequencing kit (PE Applied Biosystems).

Phylogenetic analysis

BLAST analysis was used to screen databases for sequences that were related to the isolates. 16S rDNA sequences of both isolates were determined and used for phylogenetic analysis. Sequence data for comparison were obtained from the GenBank database. Nucleotide substitution rates (*K*NUC values) were calculated[17] and phylogenetic trees were constructed using the neighbor-joining method[18]. Tree topologies were evaluated by bootstrap analysis with CLUSTAL W software[19]. The DNAML program in the PHYLIP 3.5c package[20] was used for maximum likelihood analysis with the default transition/transversion ratio of 2.000000. Sequence similarity values were determined through visual comparison and manual calculations.

DNA-DNA hybridization

DNA was isolated as described by Saito and Miura[21] with modification. DNA base composition was estimated using HPLC[22]. Levels of DNA-DNA relatedness were determined by the method of Ezaki *et al.*[23] with photobiotin and microplates.

Results and Discussion

N. vinacea is a recently proposed species[8]. Since it was first reported as a soil isolate in 2001, no other isolation of this species has been reported. Our routine laboratory testing of pathogenic *Nocardia* has revealed that 2 of the more than 500 clinical isolates of *Nocardia* in Japan[24–26] are very similar to *N. vinacea*. These observations prompted us to determine the phylogenetic position of the two strains.

Sequences of *N. vinacea* IFM 0344 and *N. anaemiae* IFM 0323^T were deposited in the DDBJ database under accession numbers AB162802 and AB162801, respectively. A database search revealed that the two isolates belong to the family *Nocardiaceae*[27] in the suborder *Corynebacterineae*. Furthermore, the phylogenetic trees (Fig. 1, 2) clarify that IFM 0344 is closely associated with *N. vinacea*. The sequence similarity value between strain IFM 0344 and *N. vinacea* IFM 10175^T was 99.8%. On the other hand, IFM 0323^T is only loosely associated with *N. vinacea* (Fig. 2): the sequence similarity value between IFM 0323^T and *N. vinacea* IFM 10175^T is 98.3%. Figure 1 shows that IFM 0323^T is also loosely associated with *Nocardia caishijiensis*, but the sequence similarity value between IFM 0323^T and *N. caishijiensis* is 98.0%, which is lower than that with *N. vinacea*.

Chemotaxonomic and morphological characteristics of these two isolates are consistent with their assignment to the genus *Nocardia*[2, 27, 28]. Both strains contained galactose and arabinose as characteristic whole-cell sugars in addition to

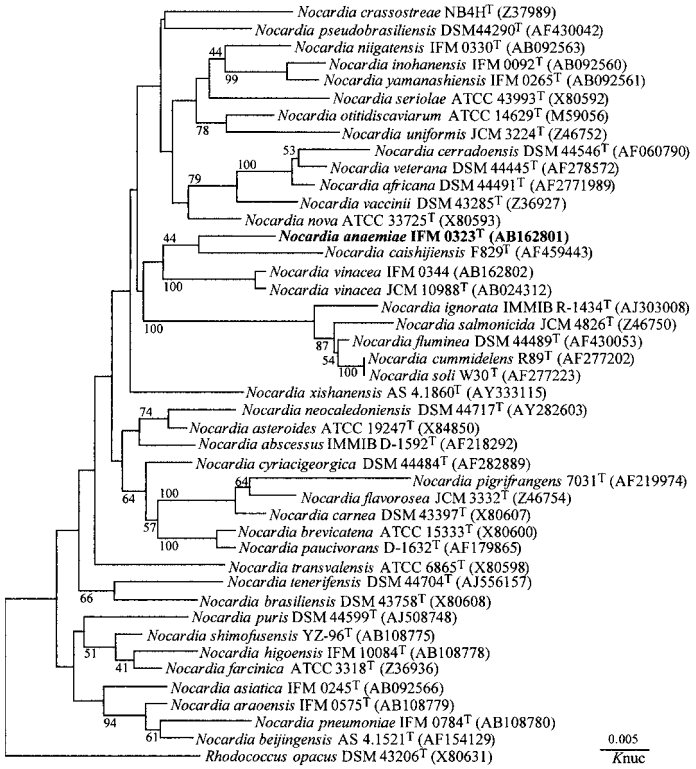

Fig. 1. Phylogenetic tree derived from 16S rDNA sequences. The tree was created using the neighbor-joining method and *K*NUC values. The numbers indicate bootstrap values for branch points. Only those values above 50% are shown.

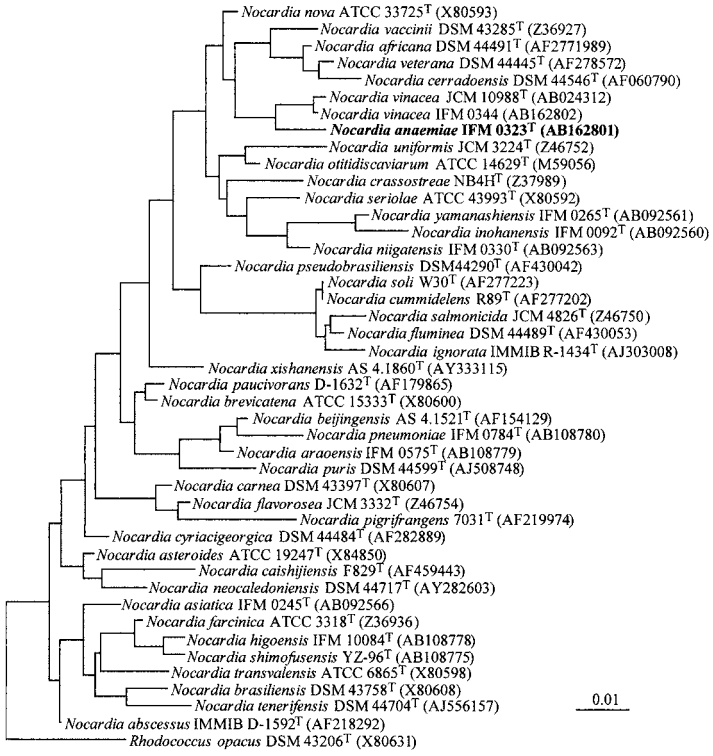

Fig. 2. Phylogenetic tree derived from 16S rDNA sequences. The tree was created using maximum likelihood analysis.

meso-diaminopimelic acid as the wall DAP. They contained mycolic acids that co-migrate (*Rf* value of about 0.47) with those extracted from the reference *Nocardia* strains. A predominant menaquinone was MK-8 ($H_{4\omega\text{-cycl}}$) (>90%).

Table 1 lists results of physiological and bio-

Table 1. Phenotypic properties tha distinguish *Nocardia anaemia* IFM 0323^T, *Nocardia vinacea* JCM 10988^T, and *Nocardia vinacea* IFM 0334 from the type strains of *Nocardia* species

	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	31
Decomposition of																															
Adenine	−	−	−	−	−	−	−	−	−	−	−	−		−	−	−	−	−		−	−		+		−	−	−	−	−	−	−
Casein	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	−	−	−	−	−	−	−	−
Hypoxanthine	+	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	+	+	−	−
Tyrosine	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	+	−	+	−	−	−	+	−	−
Urea	+	+	+	+	−	+	+	−	+	+	−	−	−	−		+	−	−		+	+		+		+	−	+	+	+	+	
Xanthine	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−
Utlization of																															
Adonitol	−	+	+																												
Arabinose	+	+	+	−		−	−					−			−	−			−	−	−	−		−	−			−		+	
Erythritol	−	−	−	−		−	−					−			−	−			−	−	−	−			−			+		−	
Galactose	+	+	+	−		−	+					−			−	−			−	+	−	−		−	+			+		+	
Glucose	+	+	+	+		+	+					+			−	+			+	+	+	−		+	+			+		+	
Inositol	−	+	+	−		−	+					−			−	−			−	−	+	−		+	−			−		−	
Maltose	−	−	−	+		−	+								−	+			+	−	−	−		−	−			+		−	
Mannose	−	−	−			−	−					+																				
Rhamnose	−	−	−	+	−	−	−	+	+	+	−	+		−	−	+	−	+	−	−	−	−	−	−	−	−	+	+	−	+	+
Sorbitol	+	+	+	−	−	−	−	+	−	−	+	+		−		−	−	−		+	−		+	+	+	−	−	+	−	−	
Citrate	+	+	+	+	−	+	+	−	+	−	−	−	−	−	−	−	−	+	−	−	−	−	+	+	+	+	−	−	−	−	−
Gluconate												−							+					−								
Growth at 37°C	+	+	+		+	+	+	+		+	+		−			+	+		+	+	+		+	+	−	−	−	+	+	+	
Growth at 45°C	−	−	−	−	+	−	−	−	−	−	−	−	−	−		+	+	−	+	−	+		−	+	−	−	−	−	−	−	+
Susceptibility test*																															
Imipenem[a]	3+	3+	3+	1+	3+	3+	−		3+		3+			3+	3+	3+				3+	−	3+	3+		3+		3+	3+			3+
Tobramycin[a]	3+	3+	3+	3+	1+	3+	3+		3+		3+			3+	3+	−				−	1+	3+	3+		3+		3+	−			−
5-Fluorouracil[b]	−	−	−	−	+	−	−		−		−			−	−	−				+	−	−	−		−		−	−			+

+, positive; −, negative.

*a, 3+ (highly susceptible, growth inhibition at 2.5 μg per disc), 1+ (slightly susceptible, growth inhibition at 10.0 μg per disc), − (not susceptible, no growth inhibition at 10 μg per disc); b, + (susceptible, growth inhibition at 30 μg per disc), −(not susceptible, no growth inhibition at 30 μg per disc).

1, IFM 0323^T; 2, IFM 0344; 3, *Nocardia vinacea* JCM 10302^T; 4, *Nocardia abscessus* DSM 44432^T; 5, *Nocardia africana* DSM 44491^T; 6, *Nocardia asteroides* ATCC 19247^T; 7, *Nocardia brasiliensis* ATCC 19296^T; 8, *Nocardia brevicatena* DSM 43024^T; 9, *Nocardia beijingensis* JCM 10666^T; 10, *Nocardia caishijiensis* JCM 11508^T; 11, *Nocardia carnea* DSM 43397^T; 12, *Nocardia cerradoensis* DSM 44546^T; 13, *Nocardia crassostreae* ATCC 70418^T; 14, *Nocardia cummidelens* DSM 44490^T; 15, *Nocardia cyriacigeorgica* DSM 44484^T; 16, *Nocardia farcinica* ATCC 3318^T; 17, *Nocardia flavorosea* JCM 3332^T; 18, *Nocardia fluminea* DSM 44488^T; 19, *Nocardia ignorata* DSM 44496^T; 20, *Nocardia nova* JCM 6044^T; 21, *Nocardia otitidiscaviarum* NCTC 1934^T; 22, *Nocardia paucivorans* DSM 44386^T; 23, *Nocardia pseudobrasiliensis* ATCC 51512^T; 24, *Nocardia puris* DSM44599^T; 25, *Nocardia salmonicida* JCM 4826^T; 26, *Nocardia seriolae* JCM 3360^T; 27, *Nocardia soli* DSM 44488^T; 28, *Nocardia transvalensis* DSM 43405^T; 29, *Nocardia uniformis* JCM 3224^T; 30, *Nocardia vaccinii* DSM 43285^T; 31, *Nocardia veterana* DSM 44445^T. Data were taken from Albuquerque *et al.*[29], Gurtler *et al.*[29]. Gurtler *et al.*[30], Hamid *et al.*[7], Kinoshita *et al.*[8], Maldonado *et al.*[31], Wang *et al.*[32], Yassin *et al.*[33–36], Zhang *et al.*[37] and this study.

chemical tests. Strain IFM 0344 and *N. vinacea* IFM 10175^T showed identical physiological characteristics, but some differences exist between strain IFM 0323^T and *N. vinacea* IFM 10175^T. For that reason, we carried out a DNA-DNA hybridization experiment between the isolates and *N. vinacea* IFM 10175^T. The DNA similarity between strains IFM 0344 and IFM 10175^T was determined as 75-83%, suggesting that strain IFM 0344 belongs to *N. vinacea*. This is the first report of human infection attributable to the *N. vinacea* strain, but we propose that the bacterium should be categorized as an opportunistic infectious group regardless of its original isolation from soil.

The DNA relatedness between strain IFM 0323^T and *N. vinacea* IFM 10175^T was 56%, suggesting that the strain should be classified as a new species. Phenotypic and phylogenetic characteristics, coupled with the data of genomic DNA relatedness levels, encourage us to propose a new *Nocardia* species: *Nocardia anaemiae* sp. nov. (type strain IFM 0323^T). During our continuing taxonomic studies on 450 nocardial

clinical isolates in Thailand and Japan, only one strain of *N. anaemiae* was isolated, suggesting a rare infectious case that is attributable to this bacterium. That case may also suggest low pathogenicity of this bacterium because it was isolated from a 73-year-old AIHA patient who had been treated with an immunosuppressive agent.

Description of *Nocardia anaemiae* sp. nov.

Nocardia anaemiae (a.na.e.mi.a. N. L. fem. n. (from Gr. fem. n. anaimia), anaemia; N. L. gen. fem. n. *anaemiae*, anaemia, referring to the disease of the patient with this condition).

Aerobic, Gram-positive, acid-fast, non-motile. Forms branched pale orange colored substrate mycelia that fragment into irregular rod-shaped elements. Produces white aerial hyphae on BHI agar medium. Dimensions of colonies are 0.2-3.0 mm after seven days at 30°C on MHII medium supplemented with 0.2% glucose.

Arabinose, galactose, glucose, citrate, and sorbitol are utilized, but adonitol, erythritol, inositol, maltose, mannose, and rhamnose are not. Hypoxanthine and urea are decomposed, but adenine, casein, tyrosine, and xanthine are not. Growth occurs at 37°C, but not at 45°C. The G+C content of the DNA is 66.2 mol%. The type strain is strain IFM 0323^T (=NBRC 100462^T, JCM 12396^T, DSM 44821^T), a clinical isolate.

References

1) Gordon RED, Barnett A, Handerhan JE, Pang CNN: *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. Int J Syst Bacteriol **24**: 54-63, 1974.
2) Goodfellow M: *Nocardia* and related genera. *In* Topley and Wilson's Microbiology and Microbial Infections, 9th edn., vol. 2, Systematic Bacteriology, pp.463-489. Edited by A. Balows and B. I. Duerden. London: Arnold: 1998.
3) Boiron P, Provost F, Chevrier G, Dupont B: Review of nocardial infections in France 1987 to 1990. Eur J Clin Infect Dis **11**: 709-714, 1992.
4) McNeil MM, Brown MJ, Georghiou PR, Allworth AM, Blacklock ZM: Infections due to *Nocardia transvalensis*: Clinical spectrum and antimicrobial therapy. Clin Infect Dis **15**: 453-463, 1992.
5) Beaman BL, Beaman L: *Nocardia* species: host-parasite relationship. Clin Microbiol Rev **7**: 213-264, 1994.
6) Kiska D, Hicks K, Pettit DJ: Identification of medically relevant *Nocardia* species with an abbreviated battery of tests. J Clin Microbiol **40**: 1346-1351, 2002.
7) Hamid ME, Maldonado L, Eldin GS, Mohamed MF, Saeed NS, Goodfellow M: *Nocardia africana* sp. nov., a new pathogen isolated from patients with pulmonary infections. J Clin Microbiol **39**: 625-630, 2001.
8) Kinoshita N, Homma Y, Igarashi M, Ikeno S, Hori M, Hamada M: *Nocardia vinacea* sp. nov. Actinomycetol **15**: 1-5, 2001.
9) Poonwan N, Kusum M, Mikami Y, Yazawa K, Tanaka Y, Gonoi T, Hasegawa S, Konyama K: Pathogenic *Nocardia* isolated from clinical specimens including those of AIDS patients in Thailand. Eur J Epidemiol **11**: 507-512, 1995.
10) Staneck JL, Roberts GD: Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl Microbiol **28**: 226-231, 1974.
11) Lechevalier MP, Lechevalier HA: The chemotaxonomy of actinomycetes. *In* Actinomycete Taxonomy. pp.227-291. Edited by Dietz and D. W. Thayer. Society for Industrial Microbiology: Virginia, 1980.
12) Miyadoh M: Identification procedure at the genus level. *In* Identification Manual of Actinomycetes. pp.9-19. Edited by S. Miyadoh, M. Hamada, K. Hotta, T. Kudo, A. Seino, K. Suzuki and A. Yokota. Business Center for Academic Societies Japan: Tokyo, 2001.
13) Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M: Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. J Chromatography **188**: 221-233, 1980.
14) Chun J, Goodfellow M: A phylogenetic analysis of genus *Nocardia* with 16S rRNA gene sequences. Int J Syst Bacteriol **45**: 240-245, 1995.
15) Kageyama A, Poonwan N, Yazawa K, Mikami Y, Nishimura K: *Nocardia asiatica* sp. nov., isolated from patients with nocardiosis in Japan and clinical specimens from Thailand. Int J Syst Evol Microbiol **54**: 125-130, 2004.
16) Kageyama A, Yazawa K, Nishimura K, Mikami Y: *Nocardia inohanensis* sp. nov., *Nocardia yamanashiensis* sp. nov., and *Nocardia niigatensis* sp. nov. isolated from clinical specimens. Int J Syst Evol Microbiol **54**: 563-569, 2004.
17) Kimura M, Ohta T: On the stochastic model for estimation of mutation distance between homologous proteins. J Mol Evol **2**: 87-90, 1972.
18) Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol **4**: 406-425, 1987.
19) Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res **22**: 4673-4680, 1994.
20) Felsenstein J: Confidence limits on phylogenies: An approach using the bootstrap. Evolution **39**: 783-791, 1985.
21) Saito H, Miura K: Preparation of transforming deoxyribonucleic acid by phenol treatment.

Biochim Biophys Acta **72**: 619-629, 1983.
22) Tamaoka J, Komagata K: Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol Lett **25**: 125-128, 1984.
23) Ezaki T, Hashimoto Y, Yabuuchi E: Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. Int J Syst Bacteriol **39**: 224-229, 1989.
24) Kageyama A, Torikoe K, Iwamoto M, Masuyama J, Shibuya Y, Okazaki H, Yazawa K, Minota S, Kroppenstedt RM, Mikami Y: *Nocardia arthritidis* sp. nov., a new pathogen isolated from a patient with rheumatoid arthritis in Japan. J Clin Microbiol **42**: 2366-2371, 2004.
25) Kageyama A, Yazawa K, Ishizawa J, Hotta K, Nishimura K, and Mikami Y: Nocardial infections in Japan from 1992 to 2001, including the first report of infection by *Nocardia transvalensis*. Eur J Epidemiol **19**: 383-389, 2004.
26) Kageyama A, Yazawa K, Nishimura K, Mikami Y: *Nocardia testaceus* sp. nov. and *Nocardia senatus* sp. nov., isolated from patients in Japan. Microbiol Immunol **48**: 271-276, 2004.
27) Stackebrandt E, Rainey FA, Ward-Rainey NL: Proposal for a new hierarchic classification system, Actinobacteria class nov. Int J Syst Bacteriol **47**: 479-491, 1997.
28) Goodfellow M, Isik K, Yates E: Actinomycete systematics: an unfinished synthesis. Nova Acta Leopold **80**: 47-82, 1999.
29) Albuquerque BEVS, Manfio GP, Maitan VR, Bataus LAM, Kim SB, Maldonado LA, Goodfellow M: *Nocardia cerradoensis* sp. nov., a novel isolate from Cerrado soil in Brazil. Int J Syst Evol Microbiol **53**: 29-33, 2003.
30) Gurtler V, Smith R, Mayall BC, Pötter-Reinemann G, Stackebrandt E, Kroppenstedt RM: *Nocardia veterana* sp. nov., isolated from human bronchial lavage. Int J Syst Evol Microbiol **51**: 933-936, 2001
31) Maldonado L, Hookey JV, Ward AC, Goodfellow M: The *Nocardia salmonicida* clade, including descriptions of *Nocardia cummidelen*s sp. nov., *Nocardia fluminea* sp. nov. and *Nocardia soli* sp. nov. Antonie Leeuwenhoek **78**: 367-377, 2000.
32) Wang L, Zhang Y, Lu Z, Shi Y, Liu Z, Maldonado L, Goodfellow M: *Nocardia beijingensis* sp. nov., a novel isolate from soil. Int J Syst Evol Microbiol **51**: 1783-1788, 2001.
33) Yassin AF, Rainey FA, Mendrock U, Brzezinka H, Schaal KP: *Nocardia abscessus* sp. nov. Int J Syst Evol Microbiol **50**: 1487-1493, 2000.
34) Yassin AF, Rainey FA, Steiner U: *Nocardia cyriacigeorgici* sp. nov. Int J Syst Evol Microbiol **51**: 1419-1423, 2001.
35) Yassin AF, Rainey FA, Steiner U: *Nocardia ignorata* sp. nov. Int. J Syst Evol Microbiol **51**: 2127-2131, 2001.
36) Yassin AF, Sträubler B, Schumann P, Shaal KP: *Nocardia puri*s sp. nov. Int J Syst Evol Microbiol **53**: 1595-1599, 2003.
37) Zhang J, Liu Z, Goodfellow M: *Nocardia caishijiensis* sp. nov., a soil actinomycete. Int J Syst Evol Microbiol **53**: 999-1004, 2003.