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Original Article

First Isolates of *Nocardia abscessus* from Humans and Soil in Japan

Akiko Kageyama[1], Katsukiyo Yazawa[1], Takuji Kudo[2], Hiroko Taniguchi[1], Kazuko Nishimura[1] and Yuzuru Mikami[1]

[1]Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8673, Japan

[2]Japan Collection of Microorganisms, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-0198, Japan

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Abstract

Nocardia abscessus, a recently established species, was isolated from patients during 2000. In the course of our taxonomic studies on 121 clinical *Nocardia* isolates in Japan 5 strains isolated from patients plus one strain isolated from soil in Japan, were found to have similar physiological characteristics to those of *N. abscessus*. Phylogenetic studies using 16S rRNA gene sequence analysis confirmed that these strains belong to *N. abscessus*. This is the first isolation report of *N. abscessus* from soil as well as from clinical samples in Japan.

Key words: *Nocardia abscessus*, 16S rDNA, first isolate in Japan

Introduction

Most *Nocardia* species are associated with human and animal infections that are difficult to diagnose because of their nonspecific clinical manifestation and histological evidence[1-3]. Nocardiosis is caused by several species of the genus *Nocardia*. Systemic disease is mostly caused by the *Nocardia asteroides* group, which contains *N. asteroides* sensu stricto, *N. farcinica* and *N. nova*[4-6]. The type species *N. asteroides* is the major cause of nocardiosis, but clinical isolates are taxonomically heterogeneous[7, 8].

N. abscessus was recently described by Yassin *et al.*[9]. During taxonomic studies, we found that five clinical isolates belong to *N. asteroides* by phenotypic characteristics, but could be differentiated from *N. asteroides* by molecular methods. Recently we also isolated from soil in Japan a *Nocardia* strain which is very similar to the five clinical isolates. Further detailed taxonomic studies indicated that these six isolates belong to *N. abscessus*. This is the first case report on the infection due to this bacterium in Japan. This paper details the taxonomical studies on these strains.

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

Materials and Methods

Bacterial strain and media

The strains IFM 0367, IFM 0663, IFM 0897, IFM 10013, IFM 10222, KNJ59 (=IFM 10308=JCM 12179), *N. abscessus* IFM 10011 (=JCM 6043, ATCC 23824), and *N. abscessus* IFM 10029^T (=DSM 44432^T), were cultured on a Muller Hinton II agar slant with 1% glucose and 1% glycerol for 1 week at 27°C. Sources of these strains are listed in Table 1. For extraction of DNA and sequencing, bacterial strains were cultured on brain heart infusion (BHI, Difco) broth for 4 days at 32°C.

Biochemical characteristics

Decomposition of adenine, casein, hypoxanthine, tyrosine, urea, and xanthine were examined by the methods of 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.*[4].

Whole cell hydrolysates were analyzed for diaminopimelic (DAP) acid isomers[10] and for

Table 1. Isolated strains.

Strain	Year	Source
IFM 0367	1992	lung biopsy of a 56-year-old man with systemic lupus erythematosus (SLE)
IFM 0663	1996	brain abscess of a 62-year-old man
IFM 0897	1999	bronchial lavage of a 69-year-old man with rheumatoid arthritis
IFM 10013	2000	septum of a 42-year-old man positive for HIV
IFM 10222	2002	septum of a 84-year-old man with lung cancer
KNJ 59	2000	soil from the Institute of Physical and Chemical Research Wako, Saitama, Japan
IFM 10011		Tsukamura, 1969[24]
IFM 10029^T		joint abscess of a 56-year-old man with a complete endoprosthesis in one of his knees

cell wall sugars[11, 12] using thin layer chromatography (TLC). Mycolic acids were analyzed as reported[13]. Menaquinones were extracted from freeze-dried biomass (500 mg) and analyzed as described by Chun & Goodfellow[14].

Preparation of genomic DNA samples

Samples were prepared using the guanidine thiocyanate method[15].

16S rDNA sequencing

16S rDNA was amplified by PCR and sequenced, using six prokaryotic 16S rDNA universal primers[15]. PCR was performed with a DNA thermal cycler (TaKaRa, Japan) using 35 cycles each consisting of denaturation at 94°C

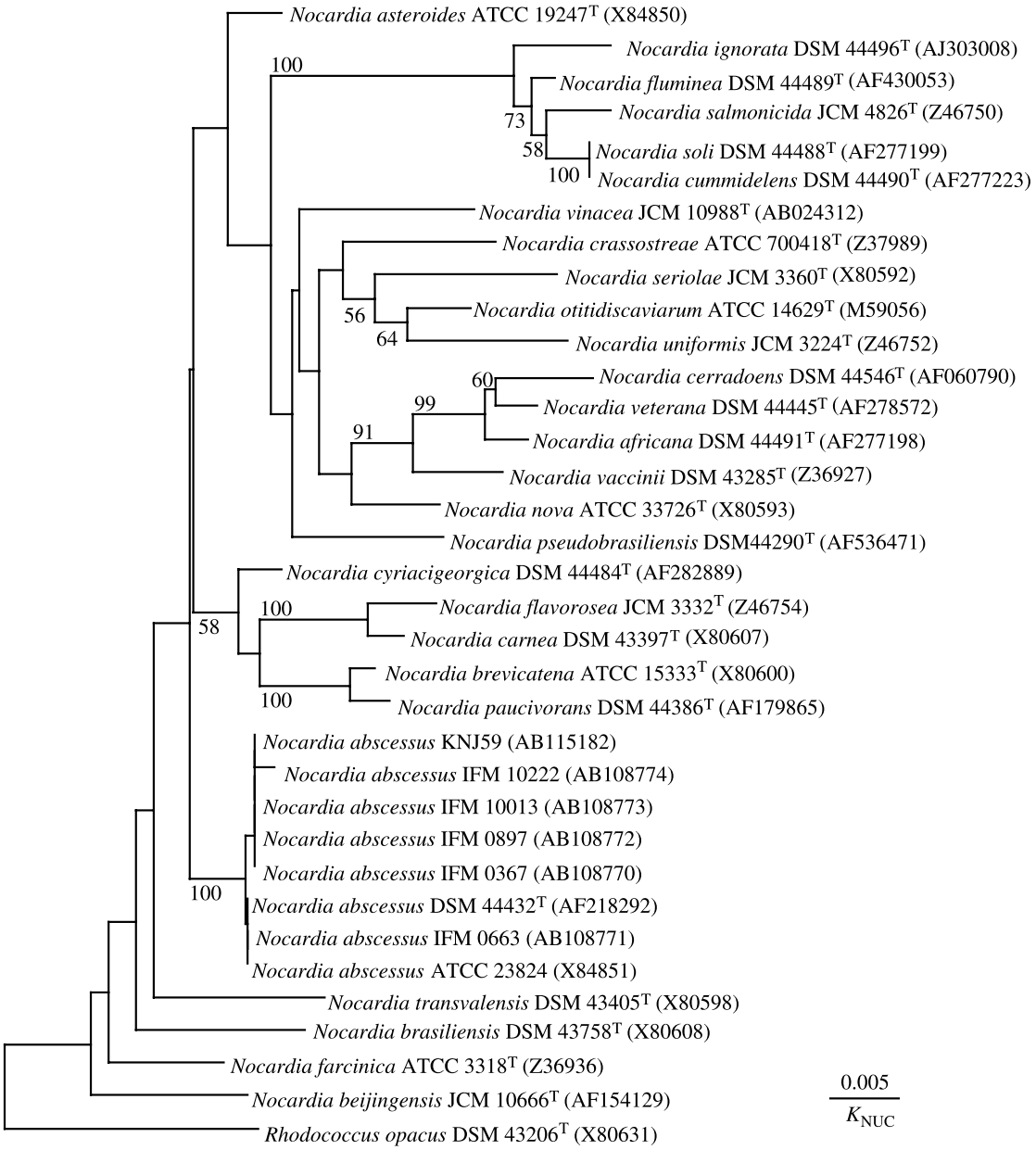

Fig. 1. Phylogenetic tree derived from 16S rDNA sequences. The tree was created using the neighbor-joining method and K_{NUC} values. The numbers indicate bootstrap values for branch points. Only values above 50% are shown.

for 60s, primer annealing at 60°C for 60s, and primer extension at 72°C for 120s. The PCR products were purified with a CENTRI-SEP COLUMN (PRINCETON SEPARATIONS). DNA sequences were determined with 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 sequence databases for strains related to the isolate. 16S rDNA sequence of all isolated strains, IFM 0367 (1432 bp), IFM 0663 (1517 bp), IFM 0897 (1508 bp), IFM 10013 (1508 bp), IFM 10222 (1481 bp), KNJ59 (1441 bp), was determined and these data were used for phylogenetic analysis. Sequence data was retrieved from the GenBank. Nucleotide substitution rates (K_{NUC} values) were calculated[16] and phylogenetic trees were constructed by the neighbor-joining method[17]. The topologies of the trees were evaluated by a bootstrap analysis using CLUSTAL W software[18].

Results and Discussion

N. abscessus is a recently characterized species[9], and since its first description in 2000, only two cases of human infection have been reported. During our routine laboratory testing of 121 pathogenic *Nocardia* strains, there were five isolated strains that were candidates of *N. abscessus*, the soil isolate KNJ59 was also found to be a candidate. These observations led us to analyze the phylogenetic position of the 6 strains. A study using more than 1300 bp of 16S rDNA region sequence analyses showed that they formed a distinctive cluster and probably belonged to *N. abscessus* (Fig. 1).

Sequence similarity values among six isolated strains and the type strain of *N. abscessus* were 99.7～100%. The phenotypic characters of the six isolated strains, type strain, and reference strain of *N. abscessus* were determined and these results were the same as that of *N. abscessus*. These studies confirmed that the six strains belong to *N. abscessus* and this is the first report of *N. abscessus* in Japan.

Since three infectious cases[9, 19, 20] due to *N. abscessus* were reported from Germany, the geographical distribution of this bacterium was previously believed to be restricted. However, our present study demonstrated that this bacterium also occurs in Japan.

During routine laboratory identification of pathogenic *Nocardia* by phenotypical and chemotaxonomical methods, the five clinical isolates were found to have a novel mycolic acid (Rf value of 0.91), which migrates with the commonly produced mycolic acid characteristic to all *Nocardia* species (Rf value of 0.47). Our survey for the presence of this novel mycolic acid (Rf value of 0.91) among reference *Nocardia* species showed that *N. abscessus* and *N. beijingensis* belong to this group (Fig. 2). Presence of this type of mycolic acid in *N. abscessus* was not reported in the original description, but this character could help to identify *N. abscessus*. We also recently reported a new species, *N. asiatica*[21], and confirmed the presence of the new mycolic acid. These three species are easily differentiated based on biochemical characteristics. Therefore, the combination of biochemical characters and detection of mycolic acid with a high Rf value is a useful characteristic to identify *N. abscessus* from other established reference *Nocardia* species, because TLC analysis is very easy and simple, compared with analysis of other chemo-taxonomical characteristics such as menaquinone.

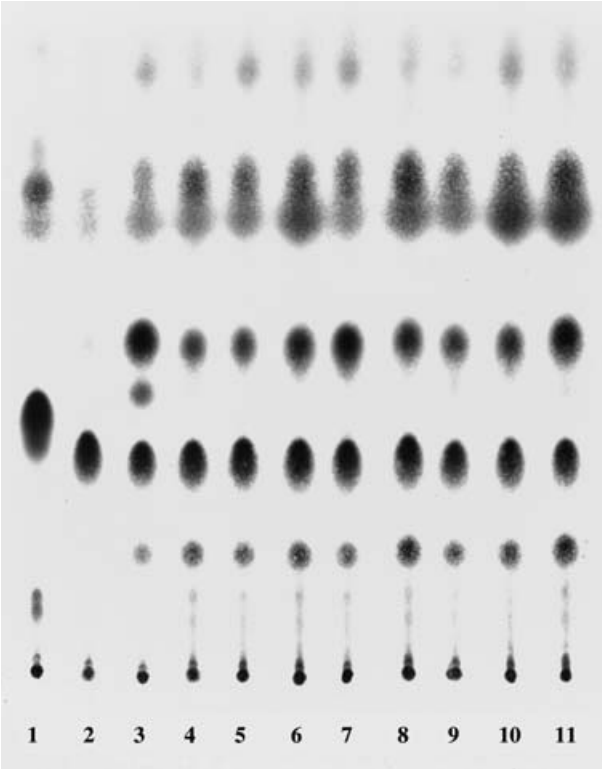

Fig. 2. Mycolic acids. 1, *Mycobacterium*; 2, typical *Nocardia*; 3, *N. beijingensis*; 4, IFM 0367; 5, IFM 0663; 6, IFM 0897; 7, IFM 10011; 8, IFM 10013; 9, IFM 10029; 10, IFM 10222; 11, KNJ59.

Many new species of the genus *Nocardia* have recently been proposed, and the range of species isolated in clinical laboratories has varied over time. Until now, *N. asteroides* sensu stricto, *N.*

farcinica, *N. nova*, *N. brasiliensis*, and *N. otitidiscaviarum* had been considered to be the predominant species.

A new species, *N. beijingensis*[22] was proposed in 2001. Originally *N. beijingensis* was isolated from soil in China, but we also isolated 18 strains from patients in Japan and demonstrated that the species is pathogenic[23]. Now *N. abscessus* must also be considered a constant constituent of pathogenic *Nocardia* in Japan.

We also recently reported the first infectious case of *N. pseudobrasiliensis* in Japan[15], and we also confirmed the presence of infections by *Nocardia transvalensis* and *Nocardia cyriacigeorgica* in Japan (unpublished data). Therefore, these experiments may show that other *Nocardia* species, which believed never to have been isolated in this country, do also exist in Japan.

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, 9 th 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) 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.
5) Javaly K, Horowitz HW, Wormser GP: Nocardiosis in patients with human immunodeficiency virus infection. Medicine (Baltimore) **71**: 128-138, 1992.
6) Mirza SH, Campbell C: Mycetoma caused by *Nocardia transvalensis*. J Clin Pathol **47**: 85-86, 1994.
7) Schaal KP, Reuterberg H: Numerical taxonomy of *Nocardia asteroids*. Zentbl Bakteriol Parasitol Infekt Hyg I Abt Suppl **6**: 53-62, 1978.
8) Orchard VA, Goodfellow M: Numerical classification of some named strains of *Nocardia asteroides* and related isolates from soil. J Gen Microbiol **118**: 295-312, 1980.
9) Yassin AF, Rainey FA, Mendrock U, Brzezinka H, Schaal KP: *Nocardia abscessus* sp. nov. Int J Syst Evol Microbiol **50**: 1487-1493, 2000.
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 A. 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: Thinlayer chromatography of methanolysates of mycolic aci-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, Sato H, Nagata M, Yazawa K, Katsu M, Mikami Y, Kamei K, Nishimura K: First human case of nocardiosis caused by *Nocardia pseudobrasiliensis* in Japan. Mycopathologia **156**: 187-192, 2002.
16) Kimura M, Ohta T: On the stochastic model for estimation of mutation distance between homologous proteins. J Mol Evol **2**: 87-90, 1972.
17) Saitou N, Nei M: The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol **4**: 406-425, 1987.
18) 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.
19) Wellinghausen N, Pietzcker T, Kern WV, Essig A, Marre R: Expanded spectrum of *Nocardia* species causing clinical nocardiosis detected by molecular methods. Int J Med Microbiol **292**: 277-282, 2002.
20) Horre R, Schumacher G, Marklein G, Stratmann H, Wardelmann E, Gilges S, De Hoog GS, Schaal KP: Mycetoma due to *Pseudoallescheria boydii* and co-isolation of *Nocardia abscessus* in a patient injured in road accident. Med Mycol **40**: 525-527, 2002.
21) 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, in press.
22) 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.
23) Kageyama A, Poonwan N, Yazawa K, Mikami Y, Nishimura K: *Nocardia beijingensis*, is a patho-

genic bacterium to humans: The first infectious cases in Thailand and Japan. Mycopathologia in press.
24) Tsukamura M: Numerical taxonomy of the genus *Nocardia*. J Gen Microbiol **56**: 265-287, 1969.