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Original Article

First Clinical Isolates of *Nocardia carnea*, *Nocardia elegans*, *Nocardia paucivorans*, *Nocardia puris* and *Nocardia takedensis* in Japan

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Abstract

Five aerobic actinomycete strains isolated from patients in Japan were assigned provisionally to the genus *Nocardia* based on morphological and physiological characteristics. The five strains, IFM 10481, IFM 0668, IFM 0901, IFM 0583 and IFM 0342, were not classified into any *Nocardia* species reported as infectious agents in Japan. Therefore, they were studied further to determine their specific taxonomic positions. Detailed chemotaxonomic and physiologic characterization and 16S rDNA sequence data of the five strains showed that they belonged to respective species of *Nocardia carnea*, *N. elegans*, *N. paucivorans*, *N. puris* and *N. takedensis*. This is the first isolation report of these five *Nocardia* species from patients in Japan.

Key words: *Nocardia* species, first isolation, Japanese isolates, pathogen

Introduction

Although nocardiosis has been considered rare, its infection incidence has recently been reported to be increasing[1, 2]. Concomitant with introduction of new genetic technologies for *Nocardia* classification, reports of new species of *Nocardia* have increased; in the years 2004 and 2005, more than 28 new species were established[1, 2]. In Japan, the following 10 *Nocardia* species had been recognized as pathogens: *N. asteroides*, *N. beijingensis*, *N. brasiliensis*, *N. cyriacigeorgica*, *N. farcinica*, *N. nova*, *N. otitidiscaviarum*, *N. pseudobrasiliensis*, *N. transvalensis* and *N. vinacea*[1, 2], and recently we added 13 new *Nocardia* species to this list: *N. anaemiae*, *N. aobensis*, *N. araoensis*, *N. arthritidis*, *N. asiatica*, *N. concava*, *N. higoensis*, *N. inohanesis*, *N. niigatensis*, *N. pneumoniae*, *N. sienata*, *N. testacea* and *N. yamanashiensis*[2-5].

Initial selection of drug therapy should incorporate species identification within the genus *Nocardia* because nocardial strains are known to show species-specific unique drug susceptibility patterns[5, 6]. For that reason, early species identifications of *Nocardia* are required to initiate effective therapy at clinical sites.

During our taxonomic studies of more than 300 strains of pathogenic *Nocardia* isolates in Japan[1], five clinical strains, IFM 10481, IFM 0668, IFM 0901, IFM 0583 and IFM 0342, were selected because they had no taxonomic similarities to those of *Nocardia* species responsible for nocardial infections that have been reported in this country. In this paper, we report taxonomic positions of these isolates based mainly on their phylogenetic information.

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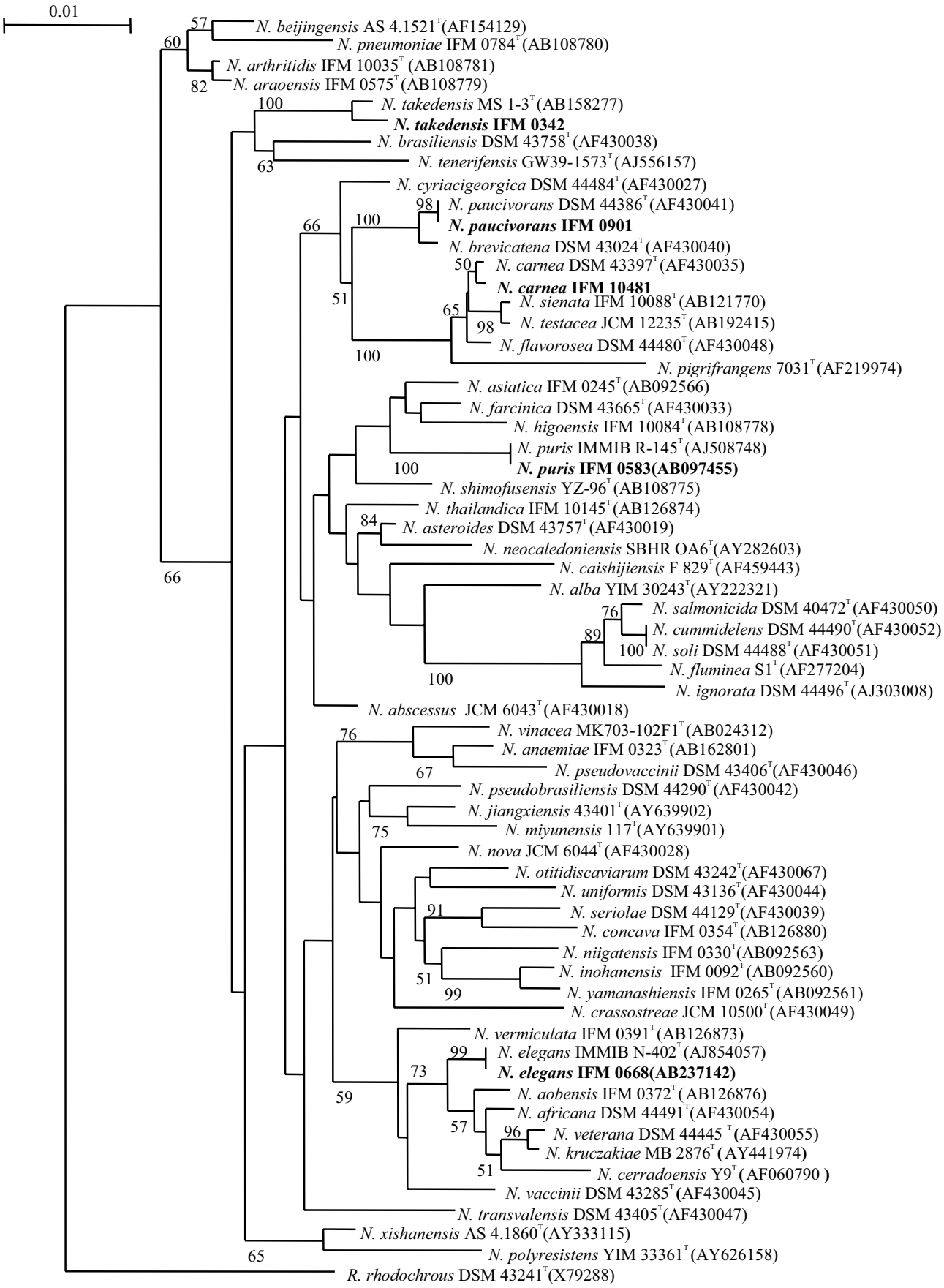

Fig. 1. Phylogenetic tree derived from 16S rDNA sequences. The tree was created using the neighbor-joining method. The scale indicates one base change per 100 nucleotide positions. Bootstrap percentages at the respective node (values <50% are not shown) were derived from 1000 replicates.

Materials and Methods

Bacterial strains and media

Strain IFM 10481 was isolated from an 84-year-old male patient with tuberculosis and bronchiectasis. Strains IFM 0668 and IFM 0901 were isolated from a 46-year-old female patient who was suspected as having bronchitis and from a 71-year-old Japanese patient with a brain tumor, respectively. Strains IFM 0583 and IFM 0342 were isolated from an 81-year-old male patient with choroiditis and from a 75-year-old patient with T-lymphoma, respectively. For extraction of DNA and DNA sequencing, the bacterial strains were cultured in brain heart infusion broth (BHI; Difco Laboratories) for 4 days at 32°C by the previously reported methods[2–5].

Biochemical, chemotaxonomic and phylogenetic characteristics

Biochemical and chemotaxonomic, characteristics were examined as reported by Kageyama *et al.*[3–5], Gordon *et al.*[6], Staneck & Roberts[7], Lechevalier & Lechevalier[8], Miyadoh[9] and Chun & Goodfellow[10]. Individual strains were tested for their ability to grow on BHI agar supplemented with 0.2% glucose with each antibiotic TRIDISK (Eiken Chemical Co. Ltd., Japan) at 32°C for 2–3 days[11]. Strains were stained by modified Kinyoun's method[12] using 0.5% sulfuric acid for determination of acid alcohol fastness. Preparation of genomic DNA samples, 16S rDNA sequencing and phylogenetic analysis were carried out as reported by Kageyama *et al.*[3–4] Phylogenetic trees were constructed by the neighbor-joining method[13]. Tree topology was evaluated by a bootstrap analysis using the CLUSTAL W software[14]. Visual comparison and manual calculation were used to determine sequence similarity values.

Results and Discussion

During our routine laboratory testing of pathogenic *Nocardia*, five strains, IFM 10481, IFM 0668, IFM 0901, IFM 0583 and IFM 0342 isolated from clinical specimens were not identified as any *Nocardia* species reported as a pathogen in Japan. This observation led us to determine their precise taxonomic positions. All the strains contained arabinose and galactose as characteristic whole cell sugars in addition to *meso*-diaminopimelic acid as a cell wall diamino acid (wall chemotype IV *sensu* Lechevalier & Lechevalier[8]). The major menaquinone of the

Table 1. Comparison of physioloical and biochemical properties of strains IFM 10481, IFM 0668, IFM 0901, IFM 0583 and IFM 0342 with those of the type strains of the corresponding *Nocardia* species.

Characteristic	*N. carnea*		*N. elegans*		*N. paucivorans*		*N. puris*		*N. takedensis*	
	IFM 0237^T	IFM 10481	IFM 10589^T	IFM 0668	IFM 10001^T	IFM 0901	IFM 10564^T	IFM 0583	IFM 10572^T	IFM 0342
Decomposition of:										
Adenine	-	-	-	-	-	-	-	-	-	-
Casein	-	-	-	-	-	-	-	-	-	-
Hypoxanthine	-	-	-	-	-	-	-	-	-	-
Tyrosine	-	-	-	-	-	-	-	-	-	-
Urea	-	-	+	+	-	-	-	-	+	+
Xanthine	-	-	-	-	-	-	-	-	-	-
Acid from(Gordon test):										
Adonitol	-	+	-	-	-	-	-	-	-	-
Arabinose	-	-	-	-	-	-	-	-	-	-
Erythritol	-	-	-	-	-	-	-	-	-	-
Galactose	+	+	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	-	-	+	+	+	+
Inositol	-	-	-	-	-	-	+	+	-	-
Maltose	-	-	-	-	-	-	-	-	-	-
Mannitol	-	-	-	-	-	-	+		-	-
Mannose	+	-	-	-	-	-	-	-	-	-
Rhamnose	-	+	-	-	-	-	-	-	-	-
Sorbitol	+	-	-	-	-	-	+	+	-	-
Utilization of:										
Citrate	-	-	-	-	-	-	+	+	-	+
Adipic acid	-	-	-	-	-	-	-	-	-	-
Gluconate	-	-	-	-	-	-	-	-	-	-
Growth at :										
37℃	+	+	+	+	+	+	+	+	+	+
45℃	-	-	+	+	+	+	+	+	-	-
Susceptibility to										
Imipenem[a]	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
Tobramycin[a]	3+	3+	2+	1+	3+	3+	3+	3+	3+	2+
5-fluorouracil[b]	-	-	+	+	-	-	-	-	+	+
Kanamycin[c]	3+	3+	3+	1+	3+	3+	-	-	3+	2+
Production of:										
β-lactamase	+	+	+	+	+	+	+	+	+	+

Scored as follows: a: 3+, highly susceptible (growth inhibition zone around 2.5 μg/disc); 2+. Moderately susceptible (growth inhibition zone around 5 μg/disc); 1+; slightly susceptible (growth inhibition zone around 10 μg/disc); b: +, susceptible (growth inhibition zone around 30 μg/disc); -, not susceptible (no growth inhibition zone around 30 μg/disc), c: 3+, highly susceptible (growth inhibition zone around 5 μg/disc); 2+, moderately susceptible (growth inhibition zone around 10 μg/disc);1+, slightly susceptible (growth inhibition zone at 30 μg/disc); -, not susceptible (no growth inhibition zone at 30 μg/disc).

three strains was MK-8 ($H_{4\omega\text{-cycle}}$). Mycolic acids from the isolates co-migrated with those from the type strains of *N. brasiliensis* and *N. asteroides*. All strains showed partial acid fastness by Kinyoun's staining method. On the basis of these results, strains IFM 10481, IFM 0668, IFM 0901, IFM 0583 and IFM 0342 were inferred to belong to the genus *Nocardia*.

Almost complete 16S rDNA sequences of the isolates were compared with those of representative species classified in the genus *Nocardia*[10].

Among the *Nocardia* species reported, the highest 16S rDNA sequence similarities of strains IFM 10481, IFM 0668, IFM 0901, IFM 0583 and IFM 0342 strains were observed with sequences of *N. carnea*, *N. elegans*, *N. paucivorans*, *N. puris* and *N. takedensis* (99.7, 100.0, 99.8, 99.9 and 99.5%), respectively. A phylogenetic tree constructed by the neighbor-joining method (Fig. 1) shows that strains IFM 10481, IFM 0668, IFM 0901, IFM 0583 and IFM 0342 form clusters with those of *N. carnea*, *N. elegans*, *N. paucivorans*, *N. puris* and *N. takedensis*, respectively.

Table 1 shows results of physiological tests in comparison with those of the respective type strains. These results supported that strains IFM 10481, IFM 0668, IFM 0901, IFM 0583 and IFM 0342 should be identified as *N. carnea*, *N. elegans*, *N. paucivorans*, *N. puris* and *N. takedensis*, respectively. To our knowledge, this is the first report of infections by these five species of *Nocardia* in Japan.

Among the five species, the type strain of *N. takedensis* is a recent isolate from soil, and no cases of infection have been reported yet. However, our present study suggests that this species should be treated as pathogenic *Nocardia*. An antibacterial antibiotic producer, *N. vinacea*, was originally isolated from soil[15], but our previous study[16] suggested that this species should be recognized as a pathogenic bacterium because it has also been isolated from Japanese patients. Present data seem to indicate that *Nocardia* species should be considered as potentially hazardous bacteria regardless of their isolation sites.

Recently we reported that *N. cyriacigeorgica* is one of the important pathogens responsible for nocardiosis in Japan[17]. Our recent studies also suggest that infections attributable to *N. transvalensis* are not rare in Japan[1]. These results suggest that nocardiosis is not rare in Japan, and further taxonomic study may lead to recognition of many other new species of *Nocardia*.

Nocardia species show species-specific drug susceptibility patterns as stated earlier[11], and therefore rapid identification of *Nocardia* species is required to initiate proper therapy. Development of simpler and more rapid identification methods is expected because the identification of *Nocardia* species in clinical laboratories is time-consuming.

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