Phylogenetic studies of *Nocardia* species based on *gyrB* gene analyses

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Phylogenetic analyses of 56 type species of *Nocardia* were conducted using the partial nucleotide sequences of the gyrase B-encoding gene (*gyrB*). The interspecies similarities of the *gyrB* gene for the 56 type species were 82.4–99.9 %, which corresponded to 270–2 nt differences in the partial gene sequences of approximately 1200 nt. In comparison with phylogenetic relationships, *gyrB* gene sequence information was generally consistent with that of 16S rRNA gene sequences with minor exceptions. However, the degree of divergence of the *gyrB* gene sequences was approximately 3.6 times greater than those of the 16S rRNA gene, suggesting a higher discriminative power of *gyrB* sequence information compared with 16S rRNA gene sequences for *Nocardia* species. The *Nocardia* type species were clustered based on *gyrB* sequence similarity values of 93.5 % and above. Among the 56 type species, 38 were distributed in 13 clusters, each comprising 2 to 7 species. The remaining 18 species were classified into an independent cluster, in which the similarity between each species and the other 55 *Nocardia* species was less than 93.5 %. Among the eight mycolic acid-containing actinomycete genera in the suborder *Corynebacterineae*, *Nocardia* was clearly differentiated from the other genera, such as *Rhodococcus*, by *gyrB* gene analyses (similarity values of *gyrB* sequences for *Nocardia* and *Rhodococcus* were 75–85 %), indicating that the *gyrB* gene is a useful alternative to the 16S rRNA gene for the determination of phylogenetic relationships between the genus *Nocardia* and the seven other actinomycete genera.

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INTRODUCTION

Members of the genus *Nocardia* are ubiquitous in the environment. They can be found worldwide as saprophytic components in fresh and salt water, soil, dust, decaying vegetation and decaying faecal deposits from animals (Brown-Elliott *et al.*, 2006). Most *Nocardia* species can cause infections in both immunocompetent and immunocompromised patients (Conville *et al.*, 2004). Infection has been considered to occur by inhalation from environmental sources, mainly soil; pulmonary disease is therefore the most common clinical form. The recent increase in immunocompromised patients has led to soaring numbers of nocardiosis patients (Kageyama *et al.*, 2004a, b; Poonwan *et al.*, 2005). Reportedly, *Nocardia*

species show a species-specific drug susceptibility pattern (Ishikawa *et al.*, 2004; Yazawa *et al.*, 1993). Therefore, early species determination is necessary to start the correct drug therapy.

Nocardia species are currently being characterized extensively by molecular analysis, in addition to chemical and biochemical analysis (Chun & Goodfellow, 1995; Hoshino *et al.*, 2007). The sequence of the 16S rRNA gene has been widely used as a molecular marker to estimate relationships in bacterial phylogeny (Goodfellow, 1998). However, the 16S rRNA gene has a low mutation rate in evolution, which occasionally makes discrimination between closely related species, such as *Nocardia abscessus* and those in the *Nocardia nova* complex, difficult (Conville *et al.*, 2004).

The gyrase B-encoding gene (*gyrB*), which encodes the β -subunit of DNA gyrase, a type II DNA topoisomerase, is present universally among Gram-positive and Gram-negative bacteria. Yamamoto & Harayama (1995) used the *gyrB* gene in the taxonomic classification of *Pseudomonas putida* and found that *gyrB* gene sequence information was more useful than that of the 16S rRNA gene because the base substitution frequency of the *gyrB* gene was much higher than that of the 16S rRNA gene.

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The GenBank/EMBL/DDBJ accession numbers for the *Nocardia gyrB* gene sequences reported in this paper are AB447397–AB447401, AB450768–AB450779 and AB450782–AB450820.

Table 1. Source of *Nocardia* type species and *R. koreensis*, and GenBank accession numbers for their *gyrB* gene sequences

R. koreensis was used as an outgroup in the neighbour-joining analysis.

Species	Strain no.	GenBank accession no.
<i>N. abscessus</i>	IFM 10029	AB447398
<i>N. africana</i>	IFM 10147	AB447399
<i>N. alba</i>	IFM 10588	AB453918
<i>N. anaemiae</i>	IFM 0323	AB447400
<i>N. aobensis</i>	IFM 0372	AB447401
<i>N. araoensis</i>	IFM 0575	AB450768
<i>N. arthritis</i>	IFM 10035	AB450769
<i>N. asiatica</i>	IFM 0245	AB450770
<i>N. asteroides</i>	IFM 0319	AB450771
<i>N. beijingensis</i>	IFM 10174	AB450772
<i>N. brasiliensis</i>	IFM 0236	AB450773
<i>N. brevicatena</i>	IFM 0283	AB450774
<i>N. caishijiensis</i>	IFM 10344	AB450775
<i>N. carnea</i>	IFM 0237	AB450782
<i>N. cerradoensis</i>	IFM 10366	AB450777
<i>N. concava</i>	IFM 0354	AB450778
<i>N. crassostreae</i>	IFM 10173	AB450779
<i>N. cummidelensis</i>	IFM 10176	AB450783
<i>N. cyriaciageorgica</i>	IFM 10235	AB450784
<i>N. elegans</i>	IFM 10589	AB450785
<i>N. exalbida</i>	IFM 0803	AB447397
<i>N. farcinica</i>	IFM 0284	AB014169
<i>N. flavourosea</i>	IFM 0851	AB450787
<i>N. fluminea</i>	IFM 10138	AB450788
<i>N. higoensis</i>	IFM 10084	AB450789
<i>N. ignorata</i>	IFM 10475	AB450790
<i>N. inohanensis</i>	IFM 0092	AB450791
<i>N. jiangxiensis</i>	IFM 10633	AB450792
<i>N. kruczkiae</i>	IFM 10565	AB450793
<i>N. miyunensis</i>	IFM 10632	AB450794
<i>N. neocaledoniensis</i>	IFM 10560	AB450795
<i>N. niigatensis</i>	IFM 0833	AB450796
<i>N. nova</i>	IFM 0290	AB450797
<i>N. otitidiscaviarum</i>	IFM 0239	AB450798
<i>N. paucivorans</i>	IFM 10001	AB450799
<i>N. pigrifrangens</i>	IFM 10533	AB450800
<i>N. pneumoniae</i>	IFM 0784	AB450801
<i>N. pseudobrasiliensis</i>	IFM 0624	AB450802
<i>N. pseudovaccinii</i>	IFM 10376	AB450803
<i>N. puris</i>	IFM 10564	AB450804
<i>N. salmonicida</i>	IFM 0849	AB450820
<i>N. seriolae</i>	IFM 0286	AB450805
<i>N. shimofusensis</i>	IFM 10331	AB450806
<i>N. sienata</i>	IFM 10088	AB450807
<i>N. soli</i>	IFM 10177	AB450808
<i>N. takedensis</i>	IFM 10572	AB450809
<i>N. testacea</i>	IFM 0937	AB450810
<i>N. thailandica</i>	IFM 10145	AB450811
<i>N. transvalensis</i>	IFM 0333	AB450812
<i>N. uniformis</i>	IFM 0856	AB450813
<i>N. vaccinii</i>	IFM 10284	AB450814

Table 1. cont.

Species	Strain no.	GenBank accession no.
<i>N. vermiculata</i>	IFM 0391	AB450815
<i>N. veterana</i>	IFM 10086	AB450816
<i>N. vinacea</i>	IFM 10175	AB450817
<i>N. xishanensis</i>	IFM 10549	AB450818
<i>N. yamanashiensis</i>	IFM 0265	AB450819
<i>R. koreensis</i>	JCM 10743	AB450821

Recently, we also reported the usefulness of *gyrB* gene information for phylogenetic studies of *Gordonia* (Kang *et al.*, 2009). In the present study, we analysed the phylogenetic relationship of 56 type species of *Nocardia* based on *gyrB* gene sequences and compared the results with those from the 16S rRNA gene.

METHODS

The bacterial strains used in this study are presented in Table 1. Type strains of each *Nocardia* species from the culture collection of the Medical Mycology Research Center, Chiba University, Japan [formerly the Research Center for Pathogenic Fungi and Microbial Toxicoses (IFM strains)] were used. They were cultured in Muller–Hinton II broth (Difco Laboratories) with 1% glucose and 1% glycerol at 30 °C for 1 week (Kageyama *et al.*, 2005). The PCR amplification procedure for the *gyrB* gene for most *Nocardia* species was applied without a DNA extraction procedure. The preparation for direct PCR amplification was as follows: 1 PCR bead (Ready-To-Go PCR beads; GE Healthcare), 22 µl dH₂O, 1 µl forward primer (20 pM), 1 µl reverse primer (20 pM) and 1 µl diluted culture were added to each test tube for PCR amplification. Fresh cultures of all *Nocardia* strains in this study were diluted with dH₂O at a concentration equal to a McFarland standard 4.0. However, when amplification was not successful, the DNA extraction was processed according to the instructions for the Isoplant II kit (Nippon Gene) for bacteria and yeast. Partial sequences of the *gyrB* gene were amplified using PCR with degenerate universal PCR primers, as described by Yamamoto & Harayama (1995), with slight modifications. The modifications of primers were based on the genome sequences of *Nocardia farcinica* (Ishikawa *et al.*, 2004). The primer sequences are presented in Table 2. The amplification reactions were performed under the following conditions: initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min and extension at 72 °C for 1.5 min, with a final extension at 72 °C for 10 min.

The PCR products were purified using ExoSAP-IT (GE Healthcare). Cycle sequencing was performed using a BigDye terminator v1.1 cycle sequencing kit (PE Applied Biosystems), as described previously (Kageyama *et al.*, 2004d). Determination of the nucleotide sequences for the PCR products was performed using an automated genetic analyser (ABI Prism 3100; PE Applied Biosystems).

The *gyrB* gene sequences determined in this study and the 16S rRNA gene sequences obtained from GenBank were aligned using CLUSTAL W (Thompson *et al.*, 1994). Nucleotide substitution rates (K_{nuc} values) were calculated (Kimura, 1980), and phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap values based on 1000 replications are listed as percentages at the branching points of the tree (Fig. 1).

Table 2. Primers used for PCR amplification and sequencing of the *gyrB* gene

<i>gyrB</i> primer	Sequence (5'→3')	Position (nt)
F1	GAGGTCGTCATGACCCAGCTGCACGCGGGCGGCAAGTTCG	355–394*
R1296	GATGTGCTGGCCGTCGACGTCGGCGTCGGCCAT	1651–1683*
UP-1	GAGGTCGTCATGACCCAGCTGCAYGCNGGNGNAARTTYGA	91–104†
UP-2r	AGCAGCGTCGAGATGTGCTGGCCRTCNACRGCNGCRTCNGTCAT	495–509†
sF1	GAGGTCGTCATGACCCAGCTGCA	355–377*
sF358	CAGGAGATGGCGTTCCTGAACA	649–670*
sF609	GTGCACACCTTCGCCAA	964–980*
sF799	AGCCGCAGTTCGAGGGCCAGAC	1154–1175*
sR495	CTTGGTCCGGTTGATGTG	850–867*
sR804	GTCTTGGTCTGGCCCTCGAACTG	1159–1181*
sR1068	GAGATCTACATCGTGGAG	1423–1440*
sR1296	GATGTGCTGGCCGTCGACGTCGGCGTCGGCCAT	1651–1683*

*Positions correspond to the *gyrB* nucleotide sequence of *N. farcinica* IFM 10152 (GenBank accession no. AP006618).

†Positions correspond to the amino acid sequences of the subunit B protein of DNA gyrase from *Escherichia coli* K-12 (GYRB_ECOLI in the Swiss-Prot database).

RESULTS AND DISCUSSION

Cultures of *Nocardia* strains were subjected to PCR amplification of the *gyrB* gene region using degenerate PCR primers. Among the 57 *Nocardia* species initially tested, the *gyrB* genes of 56 species were amplified. The *gyrB* gene of *Nocardia tenerifensis* (IFM 10554^T) was not amplified appropriately with the primers used. Amplified PCR product sizes were approximately 1200 bp, similar to those of the known partial *gyrB* sequences of other bacteria. The GenBank accession numbers of the sequences obtained are shown in Table 1.

The interspecies similarity (percentage of identical nucleotides in the sequence) range of *Nocardia gyrB* gene sequences in this study was 82.4–99.9 %, corresponding to nucleotide differences of 270–2 nt. We also obtained 16S rRNA gene sequences of the *Nocardia* species from GenBank and analysed them over a unified length (1350 bp). The interspecies similarity range of the 16S rRNA sequences was 94.4–100.0 %, corresponding to nucleotide differences of 75–0 nt (Fig. 1a). These data indicated that the divergence degree of the *gyrB* gene was approximately 3.6 (270/75 bp) times greater than that of the 16S rRNA gene.

The genus *Nocardia* belongs to the family *Nocardiaceae*, suborder *Corynebacterineae*. The suborder currently contains eight genera as the major mycolic acid-containing organisms: *Dietzia*, *Gordonia*, *Mycobacterium*, *Nocardia*, *Rhodococcus*, *Skermania*, *Tsukamurella* and *Williamsia* (Stackebrandt *et al.*, 1997; McMinn *et al.*, 2000). Table 3 shows the intergenus similarity ranges (*gyrB* and 16S rRNA gene sequences) between the studied *Nocardia* species and the other seven mycolic acid-containing genera. For the *gyrB* gene sequences, the intergenus similarities between *Nocardia* and the other genera were 68.1–87.3 %, with a mean value of 76.1 %. For the 16S rRNA gene sequences,

the similarities were 88.9–97.2 %, with a mean value of 93.1 %. These data suggested that the discriminatory power of the *gyrB* gene is approximately 3.8 times greater than that of the 16S rRNA gene for differentiating *Nocardia* species from other actinomycetes. From the study of *gyrB* gene sequences, it was also evident that the genus *Nocardia* is most closely related to the genus *Rhodococcus*, followed by *Mycobacterium* and *Gordonia* (Table 3). Because all of these mycolic acid-containing actinomycetes are mutually closely related, and because it is not easy to discriminate them by morphological and biochemical methods, the *gyrB* sequence information might help the development of a useful laboratory technique for identifying *Nocardia* and other organisms.

Phylogenetic trees based on *gyrB* and 16S rRNA gene sequences for the 56 type species of *Nocardia* studied were constructed using the neighbour-joining method with *Rhodococcus koreensis* as an outgroup (Fig. 1a, b). A similar phylogenetic tree was constructed with the amino acid sequences deduced from the *gyrB* genes (data not shown). Comparison of the phylogenetic trees based on the *gyrB* and 16S rRNA genes revealed that the two trees showed similar but not entirely identical phylogenetic relationships for the *Nocardia* species. However, most bootstrap values of the *Nocardia* species on the *gyrB* tree were significantly higher than those on the 16S rRNA gene tree.

Based on interspecies similarities of the *gyrB* gene sequences greater than 93.5 %, we grouped the 56 *Nocardia* type strains into a number of clusters (Table 4). The first 13 clusters were as follows: cluster 1, *Nocardia africana*, *Nocardia aobensis*, *Nocardia cerradoensis*, *Nocardia elegans*, *Nocardia kruczakiae*, *N. nova* and *Nocardia veterana* (similarities of 94.1–99.6 %); cluster 2, *N. abscessus*, *Nocardia arthritidis*, *Nocardia asiatica*, *Nocardia exalbida* and *Nocardia pneumoniae* (93.6–98.0 %); cluster 3, *Nocardia alba*, *Nocardia cummidelens*,

Fig. 1. Phylogenetic trees derived from the 16S rRNA gene (a) and the *gyrB* gene (b) sequences of 56 type species of *Nocardia*. The trees were created using the neighbour-joining method. The respective K_{nuc} values were 0.005 in (a) and 0.02 in (b). The numbers on the tree represent bootstrap values for the branch points. Bootstrap values greater than 50 % significance are indicated.

Table 3. Relative similarity values of the *gyrB* gene sequences of the seven other mycolic acid-containing actinomycete genera compared with *Nocardia* species

Genus	<i>gyrB</i> (%)	16S rRNA (%)
<i>Rhodococcus</i>	75.2–87.3	92.4–97.2
<i>Skermania</i>	73.8–79.8	91.4–95.1
<i>Tsukamurella</i>	73.9–80.5	92.0–95.0
<i>Gordonia</i>	72.8–82.5	90.8–94.9
<i>Mycobacterium</i>	75.5–82.0	88.9–94.6
<i>Dietzia</i>	68.1–73.3	89.7–94.4
<i>Williamsia</i>	68.5–73.0	90.8–96.2
Range (minimum–maximum)	68.1–87.3	88.9–97.2
Mean similarity*	76.1	93.1

*Mean similarities were estimated from the maximum and minimum values of each genus.

Nocardia fluminea, *Nocardia soli* and *Nocardia salmonicida* (97.1–99.4%); cluster 4, *Nocardia anaemiae* and *Nocardia vinacea* (97.0%); cluster 5, *Nocardia araoensis* and *Nocardia beijingensis* (97.1%); cluster 6, *Nocardia asteroides*, *Nocardia neocaledoniensis* and *Nocardia thailandica* (93.6–96.7%); cluster 7, *Nocardia caishijiensis* and *Nocardia ignorata* (97.9%); cluster 8, *Nocardia carnea* and *Nocardia flavovirens* (93.7%); cluster 9, *Nocardia concava* and *Nocardia seriolae* (95.5%); cluster 10, *Nocardia higoensis* and *Nocardia shimofusensis* (95.2%); cluster 11, *Nocardia inohanensis* and *Nocardia yamanashiensis* (96.9%); cluster 12, *Nocardia miyunensis* and *Nocardia vaccinii* (94.7%); cluster 13, *Nocardia sienata* and *Nocardia testacea* (96.3%). The 13 clusters formed individual clades in the phylogenetic tree based on the *gyrB* sequences. There were 18 remaining *Nocardia* type species that had *gyrB* similarities less than 93.5% when compared with the remaining species. These 18 *Nocardia* type strains were only distantly related to the other species, indicating they could easily be discriminated based on their gene sequence information in a phylogenetic study. The results confirmed that *gyrB* gene sequence information is useful for interspecies discrimination of *Nocardia* strains, as well as for intergenus discrimination.

High interspecies similarities of the *gyrB* gene sequences were found among *N. soli*, *N. cummidelensis*, *N. fluminea*, *N. salmonicida* and *N. alba*. The amino acid sequences deduced from the genes of the first four were identical, whilst the similarities of the 16S rRNA gene sequences in the four species ranged from 97.5 to 99.1%. According to a published study, however, the four species are distinguishable by, for example, different colony colours (Maldonado *et al.*, 2000). In addition, the amino acid sequences deduced from the *gyrB* genes of *N. aobensis* and *N. kruczakiae* were identical. These organisms have similar phenotypes in several biochemical tests, but differ in their

Table 4. Grouping of the 56 type species of *Nocardia* based on *gyrB* gene sequence similarity values of 93.5% or above

Clusters numbered 14–31 comprised only one species because the similarity of their respective *gyrB* sequences to the remaining *Nocardia* species was less than 93.5%.

Cluster no.	Species	Strain no.
1	<i>N. africana</i>	IFM 10147
	<i>N. aobensis</i>	IFM 0372
	<i>N. cerradoensis</i>	IFM 10366
	<i>N. elegans</i>	IFM 10589
	<i>N. kruczakiae</i>	IFM 10565
	<i>N. nova</i>	IFM 0290
	<i>N. veterana</i>	IFM 10086
2	<i>N. abscessus</i>	IFM 10029
	<i>N. arthritis</i>	IFM 10035
	<i>N. asiatica</i>	IFM 0245
	<i>N. exalbida</i>	IFM 0803
	<i>N. pneumoniae</i>	IFM 0784
3	<i>N. alba</i>	IFM 10588
	<i>N. cummidelensis</i>	IFM 10176
	<i>N. fluminea</i>	IFM 10138
	<i>N. salmonicida</i>	IFM 0849
	<i>N. soli</i>	IFM 10177
4	<i>N. anaemiae</i>	IFM 0323
	<i>N. vinacea</i>	IFM 10175
5	<i>N. araoensis</i>	IFM 0575
	<i>N. beijingensis</i>	IFM 10174
6	<i>N. asteroides</i>	IFM 0319
	<i>N. neocaledoniensis</i>	IFM 10560
	<i>N. thailandica</i>	IFM 10145
7	<i>N. caishijiensis</i>	IFM 10344
	<i>N. ignorata</i>	IFM 10475
8	<i>N. carnea</i>	IFM 0237
	<i>N. flavovirens</i>	IFM 0851
9	<i>N. concava</i>	IFM 0354
	<i>N. seriolae</i>	IFM 0286
10	<i>N. higoensis</i>	IFM 10084
	<i>N. shimofusensis</i>	IFM 10331
11	<i>N. inohanensis</i>	IFM 0092
	<i>N. yamanashiensis</i>	IFM 0265
12	<i>N. miyunensis</i>	IFM 10632
	<i>N. vaccinii</i>	IFM 10284
13	<i>N. sienata</i>	IFM 10088
	<i>N. testacea</i>	IFM 0937
14	<i>N. brasiliensis</i>	IFM 0236
15	<i>N. brevicatena</i>	IFM 0283
16	<i>N. crassostreae</i>	IFM 10173
17	<i>N. cyriacigeorgica</i>	IFM 10235
18	<i>N. farcinica</i>	IFM 0284
19	<i>N. jiangxiensis</i>	IFM 10633
20	<i>N. niigatensis</i>	IFM 0833
21	<i>N. otitidiscaviarum</i>	IFM 0239
22	<i>N. paucivorans</i>	IFM 10001
23	<i>N. pigrifrangens</i>	IFM 10533
24	<i>N. pseudobrasiliensis</i>	IFM 0624
25	<i>N. pseudovaccinii</i>	IFM 10376
26	<i>N. puris</i>	IFM 10564
27	<i>N. takedensis</i>	IFM 10572
28	<i>N. transvalensis</i>	IFM 0333

Fig. 2. Signature base sequence motif of the *gyrB* gene for discrimination of clinically important *Nocardia* species: *N. asteroides*, *Nocardia brasiliensis*, *N. farcinica*, *N. nova*, *Nocardia otitidiscaviarum* and *Nocardia transvalensis*. Asterisks indicate identical nucleotides, and dashes indicate nucleotides not present in the sequence.

growth characteristics at 45 °C (Conville *et al.*, 2004; Kageyama *et al.*, 2004c).

In contrast, although a minimal difference (similarity of 99.5%) in the 16S rRNA gene sequences has been found between *Nocardia paucivorans* and *Nocardia brevicatena* (Roth *et al.*, 2003), they could clearly be differentiated from each other by *gyrB* gene analysis, with a similarity of 92.8% in this study. Other closely related species such as *N. carnea* and *N. flavorosea*, with a high 16S rRNA gene similarity of 99.3% (Roth *et al.*, 2003), could also be distinguished by *gyrB* sequence, with 93.7% sequence similarity (79 nt difference), indicating the usefulness of sequence analysis of *gyrB* genes in the classification.

N. exalbida was grouped into cluster 2 (with *N. abscessus*, *N. arthritidis*, *N. asiatica*, *N. pneumoniae*) based on *gyrB* analysis (Table 4, Fig. 1b), although it was grouped with *Nocardia xishanensis* based on 16S rRNA gene analysis (with a similarity of 98.7%; Fig. 1a). Both *N. miyunensis* and *N. vacinii* were grouped into cluster 12 by *gyrB* gene sequences (with a similarity of 94.7%; Table 4, Fig. 1b), but the two species were separated into different clusters in the phylogenetic analysis of the 16S rRNA gene (with a similarity of 97.2%; Fig. 1a). These data showed that some discrepancies exist in the phylogenetic positions of the species based on *gyrB* or 16S rRNA gene sequences. Therefore, genetic analyses based on other genes might be helpful for establishing a robust taxonomic relationship for *Nocardia* species. The introduction of comprehensive multilocus strain typing methods might be helpful (Hanafy *et al.*, 2008).

Within the *gyrB* nucleotide sequences of approximately 1200 nt analysed in the present study, we found a species-specific region of 60 nt, starting at approximately 300 nt from the initiation codon (Fig. 2). The sequence information from this region of the *gyrB* gene might help in the design of useful PCR primers for the rapid identification of pathogenic *Nocardia* species in clinical laboratories.

The introduction of new molecular methods has had an enormous impact on the taxonomy of actinomycetes

including members of the genus *Nocardia* (Kang *et al.*, 2009). Information from 16S rRNA gene sequences has been used regularly for these studies (Brown-Elliott *et al.*, 2006). However, the divergence of 16S rRNA gene sequences within *Nocardia* species is too low to satisfy taxonomic analyses. This study therefore shows the usefulness and limits of *gyrB* gene sequencing for phylogenetic studies and species identification for members of the genus *Nocardia*.

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