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Analysis of *secA1* Gene Sequences for Identification of *Nocardia* Species

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Molecular methodologies, especially 16S rRNA gene sequence analysis, have allowed the recognition of many new species of *Nocardia* and to date have been the most precise methods for identifying isolates reliably to the species level. We describe here a novel method for identifying *Nocardia* isolates by using sequence analysis of a portion of the *secA1* gene. A region of the *secA1* gene of 30 type or reference strains of *Nocardia* species was amplified using *secA1*-specific primers. Sequence analysis of 468 bp allowed clear differentiation of all species, with a range of interspecies similarity of 85.0% to 98.7%. Corresponding 16S rRNA gene sequences of a 1,285-bp region for the same isolates showed a range of interspecies similarity of 94.4 to 99.8%. In addition to the type and reference strains, a 468-bp fragment of the *secA1* gene was sequenced from 40 clinical isolates of 12 *Nocardia* species previously identified by 16S rRNA gene sequence analysis. The *secA1* gene sequences of most isolates showed >99.0% similarity to the *secA1* sequences of the type or reference strain to which their identification corresponded, with a range of 95.3 to 100%. Comparison of the deduced 156 amino acid sequences of the SecA1 proteins of the clinical isolates showed between zero and two amino acid residue differences compared to that of the corresponding type or reference strain. Sequencing of the *secA1* gene, and using deduced amino acid sequences of the SecA1 protein, may provide a more discriminative and precise method for the identification of *Nocardia* isolates than 16S rRNA gene sequencing.

Because of the difficulty of identifying *Nocardia* isolates by phenotypic methods, sequence analysis of the 16S rRNA gene has become the "gold standard" for the identification of *Nocardia* isolates to the species level. The use of 16S rRNA gene sequencing has been instrumental in the discrimination of numerous new species of *Nocardia* in recent years, and many of these species have been shown to be clinically significant. However, the 16S rRNA gene sequences of some distinct *Nocardia* species have been shown to be quite similar; DNA-DNA hybridization studies have shown that even species with as much as 99.8% 16S rRNA gene sequence similarity (as seen with *N. veterana* and *N. kruczakiae*) can be distinct species (2, 12). The MicroSeq 500 sequencing system (Applied Biosystems, Foster City, CA) has been shown to be useful for the identification of many species of *Nocardia* (1); however, analysis of more than 500 bp may be necessary to clearly differentiate some species, depending on the degree of base divergence which is considered acceptable for conspecific isolates. Restriction endonuclease analysis using portions of the 16S rRNA gene and the 65-kDa heat shock protein gene has been used in the past for the identification of commonly isolated *Nocardia* species (4, 10). However, the usefulness of this procedure is becoming limited (8) due to the need to determine restriction fragment length polymorphisms (RFLPs) for the expanding number of described pathogenic species and the increasing number of restriction endonucleases required to make the species distinctions among these species. Therefore, sequence analysis of an alternative gene appears to be a viable adjunct to, or even a substitute for, 16S rRNA gene sequencing for the precise identification of *Nocardia* species.

The SecA1 protein is an essential component of the preprotein translocase ATPase that provides the driving force for the export of proteins across the bacterial cytoplasmic membrane (9). It has recently been shown that sufficient variability exists in the sequence of the *secA1* gene of mycobacteria to allow discrimination of 29 species (13). We describe here a novel method of distinguishing 29 species or taxa of *Nocardia*, using sequence analysis of both a portion of the *secA1* gene and the deduced amino acid sequence.

MATERIALS AND METHODS

Type and reference strains. The type and reference strains evaluated are listed in Table 1.

Patient isolates. The *secA1* gene of 40 patient isolates representing 12 species or species groups was sequenced. Isolates were obtained from patients being treated at the Clinical Center of the National Institutes of Health (15 isolates), the University of Maryland Hospital, Baltimore, Maryland (1 isolate), the Children's Hospital and Regional Medical Center, Seattle, Washington (1 isolate), Hennepin County Medical Center, Minneapolis, Minnesota (1 isolate), or the Walter Reed Army Medical Center, Washington, D.C. (1 isolate), or were isolates referred for identification to the Microbiology Laboratory of the Maryland State Department of Health and Hygiene, Baltimore, Maryland (1 isolate), ARUP Laboratories, Salt Lake City, Utah (12 isolates), or the University of Texas Health Center at Tyler, Tyler, Texas (8 isolates) (Table 2).

Identification of clinical isolates. Clinical isolates were identified to the species level using sequence analysis of the 16S rRNA gene (3).

Molecular analysis of the *secA1* gene. DNA was extracted from the type or reference strains and the clinical isolates of *Nocardia* as previously described (4). A region of the *secA1* gene (corresponding to bases 444 to 913 of the *secA1* gene sequence of *N. farcinica* IFM 10152 [7]) was amplified using *secA1*-specific primers with tails containing M13 binding sites. The sequences of the primers were (sequence of the tail is indicated in bold type): 5′ **GTA AAA CGA CGG CCA G**GA CAG YGA GTG GAT GGG YCG SGT GCA CCG 3′ and 5′ **CAG GAA ACA GCT ATG AC**G CGG ACG ATG TAG TCC TTG TC 3′ (Midland Certified Reagent Company, Crawford, Texas). PCR was performed using a reaction mixture containing 2.5 mM $MgCl_2$, 1× LightCycler-FastStart DNA Master Hyb-

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TABLE 1. Type and reference strains of *Nocardia* species used in the evaluation of the *secA1* gene sequence

Species	Type or reference strain	GenBank accession no.	
		secA1 gene	16S rRNA gene
Nocardia abscessus	ATCC BAA-279^T	DQ360260	DQ659895
Nocardia africana	DSM 44491^T	DQ360261	AY089701
Nocardia arthritidis	DSM 44731^T	DQ360262	DQ659896
Nocardia asiatica	DSM 44668^T	DQ360263	DQ659897
Nocardia asteroides	ATCC 19247^T	DQ360267	DQ659898
Nocardia asteroides drug pattern IV	ATCC 49872	DQ360265	DQ659899
Nocardia asteroides drug pattern VI[a]	ATCC 14795	DQ360266	DQ659900
Nocardia beijingensis	JCM 10666^T	DQ360268	DQ659901
Nocardia brasiliensis	ATCC 19296^T	DQ360269	DQ659902
Nocardia brevicatena	ATCC 15333^T	DQ360270	DQ659903
Nocardia carnea	DSM 43397^T	DQ360271	AF430035
Nocardia cyriacigeorgica[a]	DSM 44484^T	DQ360272	DQ659904
Nocardia elegans	DSM 44890^T	DQ360273	DQ659905
Nocardia farcinica	ATCC 3318^T	DQ360274	DQ659906
Nocardia ignorata	DSM 44496^T	DQ360275	DQ659907
Nocardia inohanensis	DSM 44667^T	DQ360276	DQ659908
Nocardia kruczakiae	ATCC BAA-948^T	DQ360277	DQ659909
Nocardia niigatensis	DSM 44670^T	DQ360278	DQ659910
Nocardia nova	ATCC 33726^T	DQ360279	DQ659911
Nocardia otitidiscaviarum	ATCC 14629^T	DQ360280	DQ659912
Nocardia paucivorans	ATCC BAA-278^T	DQ360281	DQ659913
Nocardia pseudobrasiliensis	ATCC 51512^T	DQ360282	DQ659914
Nocardia seriolae	JCM 3360^T	DQ360284	DQ659915
Nocardia sienata	DSM 44766^T	DQ360285	AB121770
Nocardia testacea	DSM 44765^T	DQ360286	AB121769
Nocardia transvalensis	ATCC 6865^T	DQ360287	DQ659916
Nocardia vaccinii	ATCC 11092^T	DQ366276	DQ659917
Nocardia veterana	DSM 44445^T	DQ360288	DQ659918
Nocardia vinacea	JCM 10988^T	DQ360289	DQ659919
Nocardia yamanashiensis	DSM 44669^T	DQ360290	DQ659920

[a] *N. asteroides* drug pattern VI and *N. cyriacigeorgica* are thought to be conspecific.

Probe (Roche, Mannheim, Germany), 1 pmol of each primer, and approximately 0.2 μg of extracted DNA and ultrapure water to a final volume of 25 μl or with PuReTaq Ready-To-Go PCR beads (GE Healthcare, Fairfield, Conn.), using 1 pmol of each primer. The DNA was denatured at 95°C for 5 min and then subjected to 35 cycles of amplification (95°C for 1 min, 60°C for 1 min, and 72°C for 1 min), followed by a 10-min extension at 72°C. Aliquots (8 μl) of the resulting PCR amplification products were electrophoresed on 2% Tris acetate-EDTA gels (SeaKem GTG; Cambrex, East Rutherford, New Jersey). The resulting bands were dissected, and the DNA was purified using the GFX PCR DNA and gel band purification kit (GE Healthcare). Cycle sequencing was performed using M13-20 forward (5′ GTA AAA CGA CGG CCA G 3′) and M13 reverse (5′ CAG GAA ACA GCT ATG AC 3′) primers (Midland Certified Reagent Company). All cycle sequencing reactions were performed with the ABI Prism BigDye Terminator cycle sequencing Ready Reaction kit (PerkinElmer Applied Biosystems, Foster City, Calif). Excess dye terminators were removed by ethanol-sodium acetate precipitation according to the guidelines of the manufacturer. Fluorescence-based sequence analysis of the extension products was performed with the ABI 3100 genetic analyzer (Applied Biosystems/Hitachi, Foster City, Calif). The resulting sequences were assembled using Lasergene SeqMan II software (DNA Star, Inc., Madison, Wis.), and sequences were aligned, amino acid sequences deduced, and phylogenetic trees prepared using the CLUSTAL W algorithm with Lasergene MegAlign software (DNA Star, Inc.).

Molecular analysis of the 16S rRNA gene. 16S rRNA gene sequences were determined as previously described (3) or were obtained from GenBank (*N. carnea* AF430035, *N. sienata* AB121770, and *N. testacea* AB12169). For sequence comparison, all sequence lengths were adjusted to match the length of the shortest sequence (1,285 bp).

Nucleotide sequence accession numbers. Partial *secA1* and 16S rRNA gene sequences of the *Nocardia* type or reference strains were deposited in GenBank under accession numbers DQ360260 to DQ360263, DQ360265 to DQ360282, DQ360284 to DQ360290, DQ366276, and DQ659895 to DQ659920. The accession numbers are listed with the respective type and reference strains in Table 1.

TABLE 2. Similarity of *secA1* gene, deduced SecA1 amino acid, and 16S rRNA gene sequences for 40 clinical isolates of *Nocardia* species compared to type or reference strains

Species	No. of isolates	% Similarity to type or reference strain (no. of base or amino acid differences)		
		secA1 gene		16S rRNA gene (DNA sequence)
		DNA sequence	Amino acid sequence	
N. abscessus	2	96.0 (19)	99.4 (1)	100 (0)
	3	96.2 (18)	99.4 (1)	100 (0)
N. asteroides drug pattern IV	1	99.8 (1)	100 (0)	99.9 (1)
	2	100 (0)	100 (0)	99.9–100 (0–1)
N. asteroides drug pattern VI	2	97.2 (13)	99.4 (1)	100 (0)
	1	99.6 (2)	100 (0)	100 (0)
	1	100 (0)	100 (0)	100 (0)
N. beijingensis	2	95.3 (22)	99.4 (1)	99.9–100 (0–1)
N. brasiliensis	1	97.0 (14)	98.7 (2)	99.7 (4)
	2	99.2 (4)	99.4 (1)	99.9 (1)
	1	99.6 (2)	100 (0)	100 (0)
N. elegans	1	97.7 (11)	98.7 (2)	100 (0)
N. farcinica	2	99.0 (5)	100 (0)	100 (0)
	3	99.6 (2)	100 (0)	99.9–100 (0–1)
N. kruczakiae	3	99.8 (1)	100 (0)	99.9 (1)
N. nova	1	99.4 (3)	100 (0)	99.5 (7)
	1	99.6 (2)	100 (0)	99.8 (3)
	2	99.8 (1)	100 (0)	99.9–100 (0–1)
N. otitidiscaviarum	1	99.8 (1)	99.4 (1)	99.9 (1)
	1	100 (0)	100 (0)	99.9 (1)
N. pseudobrasiliensis	1	99.6 (2)	99.4 (1)	100 (0)
	2	99.8 (1)	100 (0)	99.9–100 (0–1)
	1	100 (0)	100 (0)	100 (0)
N. veterana	1	99.4 (3)	100 (0)	100 (0)
	2	99.8 (1)	100 (0)	99.9–100 (0–1)

RESULTS

***secA1* gene sequences of type and reference strains.** Sequence analysis and alignment of a 468-bp region of the *secA1* gene for each of the type and reference strains of *Nocardia* species showed significant base diversity within the entire gene region among all isolates. Base divergence was significant enough to allow good separation of all strains evaluated (Fig. 1). For all type and reference strains, sequence similarity to the next most similar species ranged from 85.0 to 98.7% for the *secA1* gene, compared to 94.4 to 99.8% similarity for a 1,285-bp region of the 16S rRNA gene. Species pairs previously reported to be highly similar by 16S rRNA gene sequencing (*N. brevicatena*/*N. paucivorans*, 99.5% similar; *N. sienata*/*N. testacea*, 99.7% similar; and *N. kruczakiae*/*N. veterana*, 99.8% similar) showed greater sequence diversity with the *secA1* gene sequence, with 95.3, 95.7 and 91.9% similarity for the *secA1* gene, respectively. *N. asteroides* drug pattern type VI showed 99.1% *secA1* gene sequence similarity to the type strain of *N. cyriacigeorgica* (100% 16S rRNA gene similarity).

The *secA1* gene sequence of *N. vaccinii*, a plant pathogen

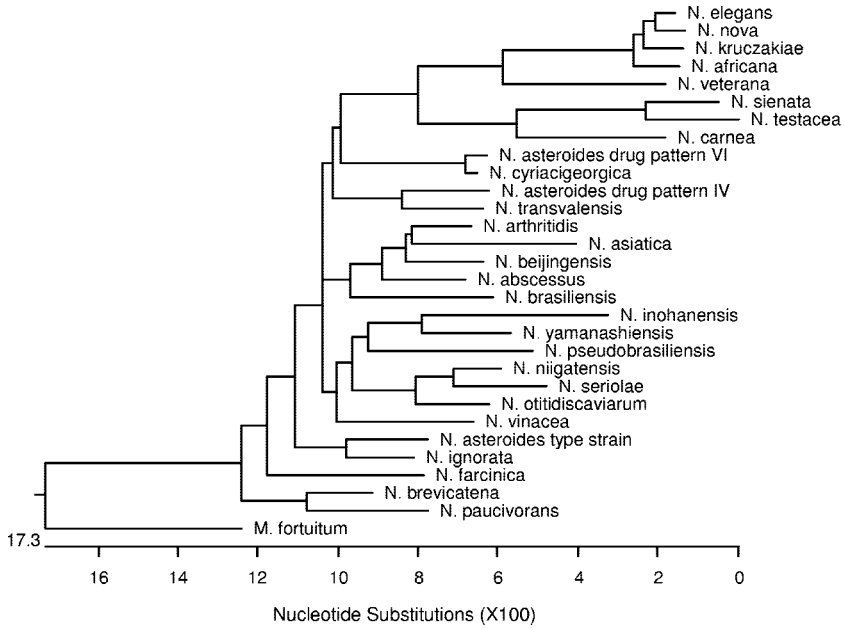

FIG. 1. Phylogenetic tree of *secA1* gene sequences of type and reference strains of *Nocardia* species.

shown to have considerable 16S rRNA gene sequence similarity to members of the *N. nova* complex (98.1 to 98.5% similarity), showed a total of 63 base insertions clustered in three areas of the gene region analyzed, resulting in an amplified region of 531 bp compared to the 468-bp region observed for other species. For *N. vaccinii*, the *secA1* gene sequence was only between 69.3% and 76.5% similar to those of other *Nocardia* type or reference strains tested (data not shown).

Alignment of the deduced amino acid sequence (comprised of 156 amino acid residues) of the 468-bp *secA1* gene region showed good separation of all type and reference strains of *Nocardia*. Each type or reference strain showed a unique amino acid sequence, with similarities among the strains ranging from 91.0% (14 amino acid differences) to 99.4% (1 amino acid difference) (Fig. 2).

***secA1* gene sequences of clinical isolates.** The *secA1* gene sequences of 24 clinical isolates belonging to *N. asteroides* drug pattern IV, *N. farcinica*, *N. kruczakiae*, *N. nova*, *N. otitidiscaviarum*, *N. pseudobrasiliensis*, and *N. veterana* showed >99.0% similarity to the *secA1* gene sequence of the type strain of the species to which they were determined to belong when analyzed by 16S rRNA gene sequencing. For clinical isolates belonging to the same species, the *secA1* gene sequence diversity was greater than the sequence diversity seen with the 16S rRNA gene sequences (Table 2). The deduced amino acid sequences of those 24 isolates belonging to the above-mentioned species showed all isolates to be between 99.4 and 100% similar to that of the type strain (zero to one amino acid difference) (Table 2).

Seven isolates determined to belong to *N. abscessus* and *N. beijingensis* by 16S rRNA gene sequence analysis showed <99.0% *secA1* gene sequence similarity to the type strains of the respective species. However, analysis of the deduced amino acid sequence for these isolates showed 99.4% similarity (one amino acid difference) to the amino acid sequences of the respective type strains.

Gene sequences of three of four isolates determined to belong to *N. brasiliensis* by 16S rRNA gene sequencing showed >99.0% and 99.4% similarity to the *secA1* gene sequence and the deduced amino acid sequence, respectively, of the *N. brasiliensis* type strain. Gene sequences of one isolate determined to be *N. brasiliensis* (99.7% 16S rRNA gene sequence similarity to the *N. brasiliensis* type strain) showed only 97.0% and 98.7% similarity to the *secA1* gene sequence and the deduced amino acid sequence (two amino acid differences), respectively, of the type strain of *N. brasiliensis*.

Two of four isolates identified as *N. asteroides* drug pattern VI (which is probably the same as *N. cyriacigeorgica*) showed >99.0% *secA1* gene sequence similarity and 100% amino acid sequence similarity to the *N. cyriacigeorgica* type strain. Two additional isolates showed 97.2% and 99.4% *secA1* gene and amino acid sequence similarities, respectively, to the type strain.

One isolate that was identified as *N. elegans* by 16S rRNA gene sequencing (100% sequence similarity) was more closely related to *N. africana* than to *N. elegans* by *secA1* gene sequencing (98.1 and 97.7% similarity, respectively); the deduced amino acid sequence of the patient isolate showed two amino acid differences compared to the amino acid sequence of the type strain of *N. elegans* and one amino acid difference compared to that of the type strain of *N. africana*.

DISCUSSION

The results presented here show that *secA1* gene sequence analysis gives good separation of all of the clinically relevant type and reference strains of *Nocardia* studied and is able to provide finer species distinctions among closely related species

FIG. 2. Phylogenetic tree of deduced SecA1 amino acid sequences of type and reference strains and clinical isolates of *Nocardia* species.

TABLE 3. Comparisons of the similarities of the 16S rRNA gene, the *secA1* gene, and the SecA1 protein of members of the *N. nova* complex[a]

Species	No. of base pair or amino acid differences (% similarity)											
	N. elegans			*N. kruczakiae*			*N. nova*			*N. veterana*		
	16S rRNA gene	*secA1* gene	SecA1 protein	16S rRNA gene	*secA1* gene	SecA1 protein	16S rRNA gene	*secA1* gene	SecA1 protein	16S rRNA gene	*secA1* gene	SecA1 protein
N. africana	6 (99.5)	8 (98.3)	3 (98.1)	10 (99.2)	12 (97.4)	3 (98.1)	22 (98.3)	12 (97.4)	3 (98.1)	8 (99.4)	39 (91.7)	4 (97.5)
N. elegans				14 (98.9)	8 (98.3)	2 (98.7)	26 (98.0)	6 (98.7)	1 (99.4)	12 (99.1)	38 (91.9)	3 (98.1)
N. kruczakiae							26 (98.0)	10 (97.9)	2 (98.7)	2 (99.8)	38 (91.9)	3 (98.1)
N. nova										26 (98.0)	38 (91.9)	3 (98.1)

[a] 16S rRNA gene, 1,285 bp; *secA1* gene, 468 bp; SecA1 protein, 156 amino acids.

than 16S rRNA gene sequence analysis. With the exception of *N. asteroides* drug pattern VI and *N. cyriacigeorgica* (see below), all other type and reference strains examined showed no more than 98.7% sequence similarity to the next most closely related species (data not shown). The most distantly related species pairs showed 85.0% sequence similarity. This range of interspecies diversity among type and reference strains is considerably larger than that observed for the same species by analysis of the 1,285-bp region of the 16S rRNA gene, which showed a range of 94.4 to 99.8% similarity (data not shown).

The reference strain of *N. asteroides* drug pattern VI and the type strain of *N. cyriacigeorgica* show 100% 16S rRNA gene sequence similarity, 99.1% *secA1* gene sequence similarity, and 100% SecA1 amino acid sequence similarity. Although results of DNA-DNA hybridization have not been published, the data presented here provide further evidence that these two strains most likely belong to the same species.

Members of the *N. nova* complex (*N. africana*, *N. kruczakiae*, *N. nova*, *N. veterana* [2], and probably *N. elegans*) are nearly indistinguishable by phenotypic methods and, in most cases, have been shown to share a very high level of 16S rRNA gene similarity (Table 3). Most of the *secA1* gene sequences of these species also show a comparatively high level of *secA1* gene sequence similarity (Table 3); *N. veterana* is the most divergent, with <92.0% similarity to any of the other species in the complex. The type strain of *N. nova* is more closely related to other members of the complex by *secA1* gene sequencing than by 16S rRNA gene sequencing (Table 3).

Analysis of the *secA1* gene may have usefulness in defining some phylogenetic relationships among *Nocardia* species. *N. vaccinii*, a pathogen of blueberries (6), has been shown to be most closely related to members of the *N. nova* complex by 16S rRNA gene sequencing. Analysis of the *secA1* gene sequence resulting from amplification with the primers noted above shows insertions of a total of 63 bases in three regions of the gene, resulting in no more than 76.5% similarity to any clinically relevant *Nocardia* species. These additional bases presumably change the size and structure of the resulting protein, reflecting the different ecologic niche of this organism.

Analysis of the *secA1* gene sequence of clinical isolates showed few isolates belonging to the same species to have identical gene sequences (data not shown). However, 27 of the 40 clinical isolates showed >99.0% similarity (zero to four base differences in a 468-bp region) to the corresponding type strain, resulting in identical deduced amino acid sequences or amino acid sequences with one residue difference (Fig. 2). Two *N. farcinica* clinical isolates showed five base pair differences compared to the *N. farcinica* type strain (99.0% similarity), with a deduced amino acid sequence identical to that of the type strain. An additional nine isolates (belonging to *N. abscessus*, *N beijingensis*, and *N. cyriacigeorgica*) showed significant *secA1* gene sequence divergence from their corresponding type strains (13 to 22 base differences); however, the deduced amino acid sequences of these isolates differed by only one amino acid residue from the type strain. This can be explained by the fact that some amino acids are determined by more than one 3-base codon. It appears that this *secA1* protein-coding gene in *Nocardia* has diversified along species lines but has not resulted in significant alteration of protein structure and, therefore, protein function, at least for the human pathogens evaluated.

With the limited number of clinical isolates of *Nocardia* studied, it appears that evaluation of the amino acid sequences of the SecA1 protein gives the most unambiguous identification. Further study with additional clinical isolates will be necessary to verify this conclusion.

Only two clinical isolates gave results with *secA1* gene and amino acid analyses that suggested that the identification based on 16S rRNA gene sequencing might be incorrect. One isolate determined to belong to *N. brasiliensis* by 16S rRNA gene sequencing (99.7% similarity of 1,285 bases to the *N. brasiliensis* type strain) showed 14 *secA1* gene base differences compared to the type strain of *N. brasiliensis* and showed a deduced amino acid sequence which differed from that of the type strain by two amino acid residues. In spite of these differences, this isolate was more similar to *N. brasiliensis* than to any other *Nocardia* species studied. One isolate identified as *N. elegans* (100% 16S rRNA gene similarity to the *N. elegans* type strain) was more closely related to the *N. africana* type strain than to the *N. elegans* type strain when the *secA1* gene sequence and the amino acid sequence were evaluated. In both of these cases, DNA-DNA hybridization is needed to determine the precise species identifications.

Phylogenetic trees of the type strains of *Nocardia* for both the 16S rRNA gene and the *secA1* gene showed similar relationships among most of the type or reference strains (Fig. 1 and 3). Some differences between the *secA1* phylogenetic tree and that of the 16S rRNA gene include the placement of *N. nova* within a clade containing other members of the *N. nova* complex in the *secA1* phylogenetic tree, the distinct placement of *N. farcinica* on a separate branch of the *secA1* tree, the separation of *N. abscessus* and the type strain of *N. asteroides*

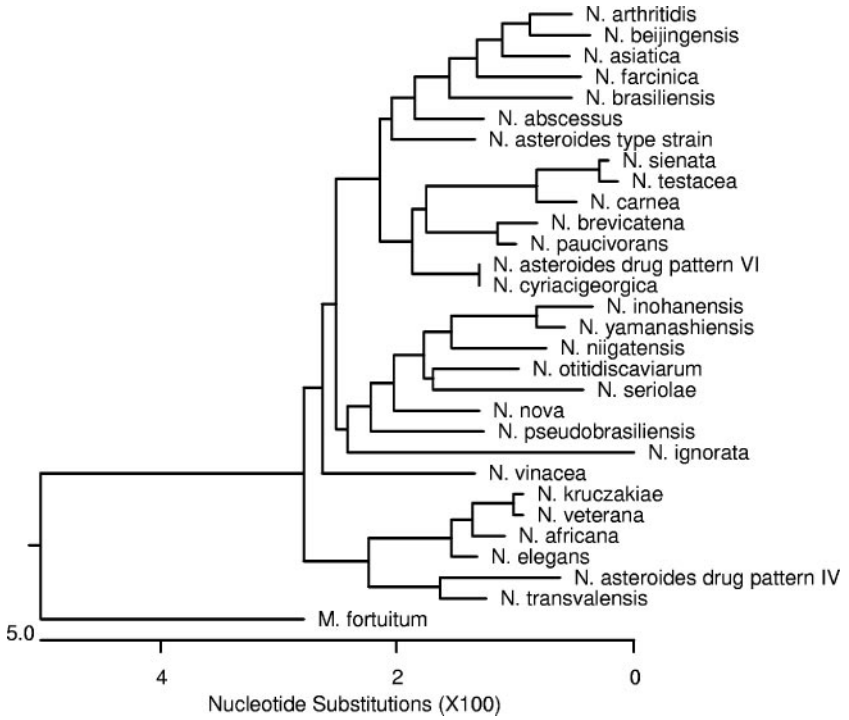

FIG. 3. Phylogenetic tree of 16S rRNA gene sequences of type and reference strains of *Nocardia* species.

on the *secA1* tree, and the different relationships of *N. ignorata*, *N. pseudobrasiliensis*, and *N. niigatensis* to other species on the two trees. These differences may reflect the phenotypic and/or pathogenic differences that exist among these species; for example, *N. nova* is phenotypically similar to other members of the *N. nova* complex (2) and *N. farcinica* is known to be particularly resistant to antibiotics and the species most likely to cause disseminated disease (11).

Unlike the 16S rRNA gene in which there has been shown to be multiple differing copies, at least in some isolates, there is little evidence to suggest that multiple different copies of the *secA1* gene exist in *Nocardia* species (5). Although it is unclear what the effect of multiple copies of the 16S rRNA gene has on accurate species identification, the use of the *secA1* gene sequence for the identification of such isolates may provide more reliable results than can be obtained with 16S rRNA gene sequencing.

Although sequence analysis of a 500-bp region of the 16S rRNA gene is adequate for the identification of numerous *Nocardia* species, extended sequence analysis may be required for the identification of some isolates from closely related species, requiring multiple sequencing reactions or more-complicated cloning and sequencing. The analysis of the 468-bp region of the *secA1* gene is sufficient for the identification of all pathogenic species of *Nocardia* analyzed, representing a more efficient and cost-effective molecular method than extended 16S rRNA gene sequencing. However, the use of this gene will require an expanded database of *secA1* gene sequences from clinical isolates to provide a true evaluation of the genetic diversity of this gene.

The use of the *secA1* gene may ultimately be most useful as part of a multigene approach to the identification of *Nocardia* isolates from human infections. Previous studies showed that the use of RFLP analysis of both the 16S rRNA and 65-kDa heat shock protein gene was useful for the identification of new and/or unusual *Nocardia* species (4). An identical identification obtained from sequence data from two or more gene targets may increase the confidence level of identifications obtained for closely related species and may allow detection of additional new species.

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