Nocardia pseudobrasiliensis sp. nov., a New Species of *Nocardia* Which Groups Bacterial Strains Previously Identified as *Nocardia brasiliensis* and Associated with Invasive Diseases

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We studied five strains of a new *Nocardia* taxon recently identified among *Nocardia brasiliensis* strains associated with invasive diseases (R. J. Wallace, Jr., B. A. Brown, Z. Blacklock, R. Ulrich, K. Jost, J. M. Brown, M. M. McNeil, G. Onyi, V. A. Steingrube, and J. Gibson, J. Clin. Microbiol. 33:1528–1533, 1995) to determine their taxonomic status. Several characteristics of these organisms, including the presence of chemotype IV cell walls, nocardomycolic acids, a predominant menaquinone similar to that of *Nocardia asteroides* ATCC 19247^T (T = type strain), and G+C contents ranging from 67 to 68 mol%, are characteristics of the genus *Nocardia*. Phylogenies based on small-subunit ribosomal DNA sequences clearly confirmed that all five strains belong to the genus *Nocardia* and occur on a single branch that is clearly distinct from *N. brasiliensis*. This branch forms a clade with *Nocardia vaccinii*, *Nocardia nova*, *Nocardia otitidiscaviarum*, and *Nocardia seriolae*. The five new strains exhibited high levels of DNA relatedness with each other, as determined by DNA-DNA hybridization experiments (S1 nuclease procedure), but not with *N. brasiliensis* strains or with strains of the four phylogenetically related *Nocardia* species mentioned above. The five new strains differ from *N. brasiliensis* in the following characteristics: mycolic acid pattern, decomposition of adenine, nitrate reduction, and antimicrobial agent susceptibilities. Therefore, we propose that these strains belong to a new species, *Nocardia pseudobrasiliensis*. The type strain is strain ATCC 51512, which was isolated from a leg abscess on a patient suffering from ulcerative colitis.

After its description in *Bergey's Manual of Systematic Bacteriology* (10) and *The Prokaryotes* (8), the genus *Nocardia* became a more well-defined taxon as a consequence of the recent transfer of *Nocardia amarae* to the genus *Gordona* (9, 16, 20) and the exclusion of *Nocardia pinensis* (3). The genus *Nocardia* now includes the following species: *Nocardia asteroides*, *Nocardia carnea*, *Nocardia brasiliensis*, *Nocardia farcinica*, *Nocardia brevicatena*, *Nocardia otitidiscaviarum*, *Nocardia nova*, *Nocardia seriolae*, *Nocardia transvalensis*, and *Nocardia vaccinii*. Most of these species cause infectious diseases in humans and animals. The majority of human nocardioses (1, 2, 4) are caused by members of the *N. asteroides* complex, which includes three species (*N. farcinica*, *N. nova*, and *N. asteroides* sensu stricto) that cannot be distinguished from each other easily (26, 27).

In contrast to *N. asteroides* sensu stricto, the other human-pathogenic *Nocardia* species, including *N. brasiliensis*, *N. otitidiscaviarum*, and *N. transvalensis*, are usually considered homogeneous (8). However, Wallace et al. (25) described a new taxon for some *N. brasiliensis* strains on the basis of taxonomic criteria, as well as clinical criteria. Taxonomically, this new taxon differs from *N. brasiliensis* in the following characteristics: decomposition of adenine, reduction of nitrate, susceptibility to ciprofloxacin and/or clarithromycin, and resistance to minocycline. Medically, members of this taxon generally in-

duce noncutaneous nocardioses, such as a pulmonary nocardiosis, a central nervous system nocardiosis, or a systemic nocardiosis, while *N. brasiliensis* sensu stricto is isolated mainly from cutaneous sites (25).

To establish more precisely the taxonomic status of the new taxon and to distinguish it from *N. brasiliensis*, we studied four of the strains used by Wallace et al. (25) and one strain isolated in France in 1992 from a patient with fatal systemic nocardiosis. The phylogenetic positions of these strains were determined precisely by comparing small-subunit ribosomal DNA (rDNA) sequences. We then determined the genomic DNA base compositions, as well as the levels of DNA relatedness among the isolates and the levels of DNA relatedness with other *Nocardia* species. On the basis of the data which we obtained, we propose that a new species, *Nocardia pseudobrasiliensis*, should be established.

MATERIALS AND METHODS

Strains, media, and growth conditions. We studied one strain (strain CIP 104598) that was isolated in the Laboratory of Bacteriology of Nice (Nice University Hospital, Nice, France) and was submitted for identification to the National Reference Center for Mycosis and Antifungal Agents, Pasteur Institute, Paris, France. The other four strains which we used (strains ATCC 51511 [= CIP 104599], ATCC 51512^T [= CIP 104600] [T = type strain], N249 [= CIP 104601], and N649 [= CIP 104602]) were included in a previous study (25). All of these strains were isolated from human clinical sources (Table 1).

All of the strains were stored either freeze-dried or on Bennett agar (13) covered with paraffin oil and were subcultured at 32°C on fresh Bennett agar or blood agar.

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TABLE 1. Levels of DNA relatedness between *N. pseudobrasiliensis* and other *Nocardia* species

Strain ^a	Source	G+C content (mol%)	DNA relatedness to strain ATCC 51512 ^T	
			% ^b	ΔT_m (°C)
<i>N. pseudobrasiliensis</i> ATCC 51511 (= CIP 104599)	Abscess on buttocks		80	0.5
<i>N. pseudobrasiliensis</i> ATCC 51512 ^T (= CIP 104600 ^T)	Leg abscess	67.1	100	0
<i>N. pseudobrasiliensis</i> N249 (= CIP 104601)	Brain abscess	67.4	88	3.0
<i>N. pseudobrasiliensis</i> N649 (= CIP 104602)	Sputum		90	0.5
<i>N. pseudobrasiliensis</i> CIP 104598	Blood	68.4	60	4.5
<i>N. brasiliensis</i> ATCC 19296 ^T			11	
<i>N. nova</i> ATCC 33726 ^T			12	
<i>N. otitidiscaviarum</i> ATCC 14629 ^T			11	
<i>N. seriolae</i> ATCC 43993 ^T			7	
<i>N. vaccinii</i> ATCC 11092 ^T			14	

^a ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection Institut Pasteur, Paris, France.^b Level of relative binding at 65°C.

Biochemical characteristics and susceptibility tests. Biochemical and susceptibility tests were performed with isolate CIP 104598 by using previously described methods (25).

Cell wall analysis. Cells were hydrolyzed and menaquinones were extracted and purified by using the procedure of Page et al. (17). The purified menaquinones were separated by reverse-phase high-performance liquid chromatography (HPLC) with a Nucleosil C18 (5 µm) column (25 cm by 4.5 mm [internal diameter]) and were eluted with methanol-isopropyl ether (3:1, vol/vol), as described by Tamaoka et al. (24). The menaquinones were detected with a UV monitor (Waters 990 photodiode array detector; Millipore Corp., Milford, Mass.) at the emission wavelength (230 to 240 nm) and were identified by their R_f values and their UV spectra. Standard menaquinones (MK-7 to MK-12) were kindly provided by Hoffman-la-Roche, Ltd., Basel, Switzerland, while di- and tetrahydrogenated menaquinones were prepared from reference strains (5). Menaquinones from *N. asteroides* ATCC 19247^T were also included in the HPLC pattern comparison.

The mycolic acid *p*-bromophenacyl ester patterns of isolate CIP 104598 were determined by the HPLC method as modified by Jost and Dunbar (14).

DNA-DNA hybridization. The cultures used for DNA extraction were grown for 72 h at 30°C in 500 ml of brain heart broth (bioMérieux). Cells were harvested by centrifugation, and the purity of each preparation was checked by growing it on sheep blood-containing Trypticase soy agar for 72 h at 30°C. The DNA was extracted as described previously (18), except that 10 mg of proteinase K (Sigma)

and 20 mg of sodium dodecyl sulfate were added to each 1 ml of lysozyme-treated bacteria. The purity of each preparation was ascertained by determining the ratio of A_{280} to A_{260} and the ratio of A_{230} to A_{260} . Hybridization experiments in which labeled DNA and fragmented DNA preparations were used were performed at 65°C for 16 h in 0.42 M NaCl by using the S1 nuclease-trichloroacetic acid method (11, 18). The denaturation temperature (the temperature at which 50% of the reassociated DNA was hydrolyzed by S1 nuclease) was determined in 0.2 M NaCl by the method of Crosa et al. (6). ΔT_m was the difference between the denaturation temperature of a homoduplex (in a homologous reaction) and the denaturation temperature of a heteroduplex (in a heterologous reaction). The DNA similarity results for each pair given below are the means of the values obtained in three separate DNA hybridization experiments.

DNA base composition. The guanine-plus-cytosine (G+C) contents of strain ATCC 51512^T, CIP 104598, and N249 DNAs were determined by using a capillary electrophoresis method and the same DNA preparations that were used for the DNA-DNA hybridization experiments. After extraction and purification on cesium chloride gradients, the DNA was hydrolyzed by the DNase I-nuclease P1 method of Gehrke et al. (7). A Waters Quanta 4000 system (Millipore) was used, and data analysis was performed by using Waters Baseline 810 software (Millipore). The deoxynucleotides were separated by using a 16-kV positive voltage and a fused-silica capillary column (total length, 54 cm; inside diameter, 50 µm; distance between injection and detection parts, 47 cm; Beckman Instruments, Palo Alto, Calif.). Samples were loaded by hydrostatic injection (10-cm height for

TABLE 2. Similarity matrix based on small-subunit rDNA sequences

Strain	Nucleotide sequence accession no.	% Similarity ^a															
		<i>N. pseudobrasiliensis</i> ATCC 51512 ^T	<i>N. pseudobrasiliensis</i> N249	<i>N. pseudobrasiliensis</i> N649	<i>N. pseudobrasiliensis</i> CIP 104598	<i>N. asteroides</i> ATCC 23824	<i>N. asteroides</i> ATCC 19247 ^T	<i>N. brasiliensis</i> ATCC 19296 ^T	<i>N. brevicatena</i> ATCC 15333 ^T	<i>N. carnea</i> ATCC 6847 ^T	<i>N. farcinica</i> ATCC 3318	<i>N. nova</i> ATCC 33726 ^T	<i>N. otitidiscaviarum</i> ATCC 14629 ^T	<i>N. seriolae</i> ATCC 43993 ^T	<i>N. transvalensis</i> ATCC 6865 ^T	<i>N. vaccinii</i> ATCC 11092 ^{Tb}	
<i>N. pseudobrasiliensis</i> ATCC 51511	X84856	99.9	99.4	100.0	99.5	97.9	97.4	96.9	97.6	96.9	96.2	98.2	97.9	96.7	96.8	96.8	
<i>N. pseudobrasiliensis</i> ATCC 51512 ^T	X84857		99.5	99.9	99.5	97.8	97.3	96.9	97.6	96.9	96.1	98.1	98.0	96.8	96.7	96.7	
<i>N. pseudobrasiliensis</i> N249	X84854			99.4	99.5	97.3	97.3	96.9	97.3	96.9	96.2	98.0	98.2	97.0	96.7	96.5	
<i>N. pseudobrasiliensis</i> N649	X84855				99.5	97.9	97.4	96.9	97.6	96.9	96.2	98.2	97.9	96.7	96.8	96.8	
<i>N. pseudobrasiliensis</i> CIP 104598	X84852					97.3	97.2	96.9	97.5	96.6	96.2	98.3	98.2	96.9	96.8	96.6	
<i>N. asteroides</i> ATCC 23824	X84850						98.9	97.6	97.6	97.6	97.6	97.4	96.9	96.5	97.3	96.7	
<i>N. asteroides</i> ATCC 19247 ^T	X84851							97.9	98.2	97.9	98.2	97.2	97.0	96.3	97.9	96.6	
<i>N. brasiliensis</i> ATCC 19296 ^T	X80591								96.6	96.6	98.0	96.4	96.1	95.8	97.4	95.6	
<i>N. brevicatena</i> ATCC 15333 ^T	X80600									97.9	96.6	97.3	97.2	96.9	97.0	97.5	
<i>N. carnea</i> ATCC 6847 ^T	X80602										96.3	96.3	96.6	96.1	96.5	96.3	
<i>N. farcinica</i> ATCC 3318	X80595											96.2	96.0	95.3	97.9	96.3	
<i>N. nova</i> ATCC 33726 ^T	X80593												98.5	97.4	97.2	97.9	
<i>N. otitidiscaviarum</i> ATCC 14629 ^T	X80599													98.0	96.8	97.4	
<i>N. seriolae</i> ATCC 43993 ^T	X80592														95.7	97.0	
<i>N. transvalensis</i> ATCC 6865 ^T	X80598															96.6	

^a Values were determined by using all nucleotides that were aligned without ambiguity (see the text).^b The nucleotide sequence accession number for *N. vaccinii* ATCC 11092^T is X80597.

FIG. 1. Phylogenetic positions of *Nocardia* species within the CMN group (20). The topology shown is part of a tree obtained by a neighbor-joining method and summarizes the results of phylogenetic analyses in which three different methods were used. The outgroups used to root the tree were representatives of the different genera belonging to the family *Pseudonocardiaceae*, the genus *Saccharothrix*, and the genus *Actinokineospora*. The double asterisks indicate branches that were also identified by a maximum-likelihood method ($P < 0.01$). Values above the lines (expressed as percentages; only values greater than 50% are shown) indicate branches that were also found on the most parsimonious tree; these values indicate how the branches were supported by the bootstrap analysis results (heuristic search). Bar = number of accumulated changes per nucleotide. The numbers in parentheses are nucleotide sequence accession numbers. The sequences of strains ATCC 51512^T and ATCC 19247^T were determined in this study. The other sequences were obtained from the EMBL database.

40 s) and were analyzed by UV detection at 254 nm. The buffer used for electrophoresis was a 35 mM sodium tetraborate solution adjusted to pH 9.45. Between runs, the capillary column was washed with 0.1 M sodium hydroxide for 3 min and distilled water for 3 min and then reconditioned with the running buffer for 2 min. The amount of each of the four nucleotides was determined by using a calibration curve established with nucleotide standards (0 to 250 μ mol/liter; Sigma Chemical Co., St. Louis, Mo.). Phage lambda DNA (G+C content, 49.858 mol%) was used as a control to determine the accuracy of the method.

Small-subunit rDNA gene sequencing. A 100-ng portion of DNA was used in a PCR to amplify the small-subunit rDNA genes. A pair of primers corresponding to positions 8 to 28 and 1491 to 1508 on the *Escherichia coli* small-subunit rDNA sequence was used in this analysis. The procedures used for amplification and direct sequencing of PCR products have been described previously (21).

Phylogenetic analysis. The phylogenetic data described below were obtained (i) by using successive alignment and phylogeny procedures and (ii) by reinvestigating deep branching patterns after close relationships were determined (21). A neighbor-joining method (22) was used for the preliminary analysis; the resulting topologies were investigated further by using maximum-likelihood and maximum-parsimony methods. For the maximum-likelihood analyses we used the *fdnaml* program rewritten by G. J. Olsen (University of Illinois, Urbana) and compiled on a Hewlett-Packard model 700 workstation, while the maximum-parsimony analyses were performed by using the PAUP program for Macintosh computers (23). The robustness of each topology was evaluated under maximum-parsimony conditions through 100 bootstrap replications (heuristic search). Trees were constructed by using the *njplot* program for Macintosh computers developed by M. Gouy (URA 243, Centre National de la Recherche Scientifique, Université Claude Bernard, Lyon, France), which allows transformation of a formal tree representation (Newick's format) into MacDraw drawings. Only topologies that were found to be similar by all three methods were considered

true trees. In recent theoretical work Kim has demonstrated that agreement of the results of all three methods which we used is a strong indication that the correct phylogeny has been determined (15).

Nucleotide sequence accession numbers. The nucleotide sequences which we determined have been deposited in the EMBL sequence database under accession numbers X84850 (*N. asteroides* ATCC 19247^T), X84851 (*N. asteroides* ATCC 23824), X84852 (*N. pseudobasiliiensis* CIP 104598), X84854 (*N. pseudobasiliiensis* N249), X84855 (*N. pseudobasiliiensis* N649), X84856 (*N. pseudobasiliiensis* ATCC 51511), and X84857 (*N. pseudobasiliiensis* ATCC 51512^T).

RESULTS AND DISCUSSION

As shown in Table 2, an analysis of the small-subunit rDNA sequences revealed that the levels of similarity were more than 99.4% when we compared the sequences of pairs of the five strains which we studied. General phylogenetic analyses revealed that these five strains belonged to a robust monophyletic unit that included all of the *Nocardia* and *Rhodococcus equi* strains belonging to one branch of the CMN group (20) whose sequences were available (Fig. 1). This finding was obtained with all of the methods which we used (neighbor joining, maximum likelihood, parsimony) and was supported by a bootstrap value of 75%. When the maximum-likelihood and parsimony methods were used, *R. equi* appeared to be a deep branch related to the genus *Nocardia*. In order to determine

FIG. 2. Unrooted tree showing the phylogenetic position of *N. pseudobrasiliensis* within the genus *Nocardia*. The results of neighbor-joining, parsimony, and maximum-likelihood analyses were combined, as described in the legend to Fig. 1. The *N. pseudobrasiliensis*, *N. vaccinii*, *N. nova*, *N. otitidiscaviarum*, and *N. seriolae* strains formed a monophyletic unit that was identified by all three methods and by a bootstrap value of 71%. The numbers in parentheses are nucleotide sequence accession numbers. The sequences of strains ATCC 51512^T, CIP 104598, N249 (N249), N649 (N649), ATCC 51511, ATCC 19247^T, and ATCC 23824 were determined in this study. The other sequences were obtained from the EMBL database.

the relationships among *Nocardia* species, we performed phylogenetic analyses with the five *N. pseudobrasiliensis* strains, all of the *Nocardia* species, and *R. equi* (as the outgroup). The topology obtained with the three phylogenetic methods clearly revealed that the type strain of *N. brasiliensis* and the new taxon should be separated (Fig. 2). All five *N. pseudobrasiliensis* strains formed a very tight monophyletic cluster with *N. vaccinii*, *N. nova*, *N. otitidiscaviarum*, and *N. seriolae*, as determined by all three methods (Fig. 2). In conclusion, the results of all of the phylogenetic analyses clearly demonstrated that the five *N. pseudobrasiliensis* strains belonged to the genus *Nocardia* on a single branch that was clearly distinct from *N. brasiliensis* and from all other *Nocardia* species.

The results of DNA-DNA hybridization experiments confirmed that the levels of similarity between strain ATCC 51512^T DNA and the DNAs of strains ATCC 51511, N249, and N649 were high, and the ΔT_m values ranged from 0.5 to 3°C (Table 1). The level of relatedness between the DNAs of strains ATCC 51512^T and CIP 104598 was lower (60%; ΔT_m , 4.5°C). However, strain CIP 104598 should probably be con-

sidered a member of the same species as the other four strains on the basis of the criteria for defining a species established by the Ad Hoc Committee on Reconciliation of Approaches to Bacterial Systematics (28), the high levels of relatedness of the rDNA sequences, and the similar phenotypes. The fact that these strains represent a new *Nocardia* species was demonstrated by the finding that the levels of DNA relatedness with the type strain of *N. brasiliensis* (which has similar phenotypic characteristics) and the four other *Nocardia* species whose small-subunit rDNA sequences are most closely related (Fig. 2) (*N. vaccinii*, *N. nova*, *N. otitidiscaviarum*, and *N. seriolae*) were less than 15% (Table 1).

On the basis of chemotaxonomic characteristics the new species clearly belongs to the genus *Nocardia*. Its cell wall contained the components found in cell wall type IV bacteria (*meso*-diaminopimelic acid, arabinose, and galactose) and mycolic acids. As determined by HPLC, these mycolic acids were similar to those found in members of the genus *Nocardia*. Strain CIP 104598 had an HPLC mycolic acid ester pattern similar to the pattern described previously for strains N249,

TABLE 3. Phenotypic characteristics used to differentiate *N. pseudobasiliiensis* from other *Nocardia* species^a

Characteristic	<i>N. pseudobasiliiensis</i>	<i>N. brasiliensis</i>	<i>N. asteroides</i> complex ^b	<i>N. brevicatena</i>	<i>N. cuneae</i>	<i>N. otitidiscavarium</i>	<i>N. seriolae</i>	<i>N. transvalensis</i>	<i>N. vacinii</i>
Decomposition of:									
Adenine	+	-	-	-	-	-	-	v	-
Casein	+	+	-	-	-	-	-	-	-
Hypoxanthine	+	+	-	-	-	+	-	+	-
Tyrosine	+	+	-	-	-	-	-	-	-
Xanthine	-	-	-	-	-	+	-	v	-
Acid produced from:									
Ribitol	-	-	-	-	-	-	-	+	-
L-Arabinose	-	-	-	-	-	v	-	-	+
Erythritol	-	-	-	-	-	-	-	+	-
D-Galactose	+	+	v	-	+	-	-	+	+
D-Glucose	+	+	+	-	+	+	+	+	+
Inositol	+	+	-	-	v	+	-	v	v
Mannitol	+	+	-	-	+	v	-	v	-
L-Rhamnose	-	-	v	-	-	-	-	-	v
D-Glucitol	-	-	-	-	+	-	-	v	v
Trehalose	+	+	v	+	+	v	-	v	v
Production of:									
Nitrate reductase	-	+	+	-	+	+	ND	+	+
Urease	+	+	+	-	-	+	-	+	+

^a Data from references 10 and 24. +, 90% or more of the strains are positive; -, 10% or less of the strains are positive; v, 11 to 89% of the strains are positive; ND, not determined.

^b The *N. asteroides* complex includes *N. nova*, *N. farcinica*, and *N. asteroides* sensu stricto, three validly described species whose physiological characteristics are similar.

N649, ATCC 51511, and ATCC 51512^T (25). Two early peaks in this pattern (peaks A and B) were not found in any of the *N. brasiliensis* sensu stricto strains. All of the strains also contained tetrahydrogenated menaquinones with eight isoprene units as their major isoprenologs, but a distinction between MK-8(H₄) and MK-8(H₄, ω-cycl) was not possible because of the experimental design of the HPLC method used. However, all of the strains produced the same HPLC menaquinone pattern as *N. asteroides* ATCC 19247^T, which contains MK-8(H₄, ω-cycl). Considering all of the data, MK-8(H₄, ω-cycl) is probably present in the strains which we examined, but this remains to be proved by adequate techniques (12). All of these chemotaxonomic characteristics, as well as the G+C contents of the DNAs (Table 1), are consistent with the description of the genus *Nocardia* (8, 10).

The sites of isolation, phenotypic characteristics, and patterns of susceptibility to antibiotics of strain CIP 104598 and the other strains belonging to the new taxon defined by Wallace et al. were similar (25). Finally, the five strains which we studied were similar to *N. brasiliensis* in some characteristics (decomposition of casein, tyrosine, xanthine, and hypoxanthine) but not in others (decomposition of adenine and nitrate reductase activity) (Table 3). These strains were susceptible to ciprofloxacin and resistant to minocycline.

In conclusion, the results of physiological tests, tests to determine susceptibility to antibiotics, mycolic acid analyses, and genomic analyses (DNA base composition determinations, DNA-DNA hybridization tests, and phylogenetic analyses of small-subunit rDNA sequences) indicated that the five invasive

isolates which we studied (four strains isolated in the United States and one strain isolated in France) are members of a new *Nocardia* species, for which we propose the name *Nocardia pseudobasiliiensis*.

Description of *Nocardia pseudobasiliiensis* sp. nov. *Nocardia pseudobasiliiensis* (pseu.do.bra.si.li.en'sis. Gr. adj. *pseudes*, false; M.L. adj. *brasiliensis*, pertaining to Brazil, a specific epithet; M.L. adj. *pseudobasiliiensis*, a false *brasiliensis*). The description below is based on data from this study and the study of Wallace et al. (25).

Strains are orange-tan to tan. The cells have typical *Nocardia* micromorphology; branched vegetative hyphae (diameter, 0.5 to 1.2 μm) fragment into bacteroid or rod-shaped to coccoid, nonmotile elements. Aerial mycelium is produced. Endospores, sporangia, sclerotia, and synnemata are not formed. No soluble pigment is produced. The cells are strictly aerobic and have an oxidative type of metabolism. They exhibit catalase, alkaline phosphatase, butyrate esterase, caprylate esterase, leucine arylamidase, acid phosphatase, phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, and *N*-acetyl-β-glucosaminidase activities.

Cells are gram positive and acid fast or partially acid fast as determined by the modified Kinyoun method (1% sulfuric acid decolorization), but they are not acid fast as determined by the standard Kinyoun method. Growth occurs at 37°C but not at 10 or 43°C after 2 weeks. Cells are resistant to lysozyme.

Adenine, casein, hypoxanthine, and tyrosine are decomposed, but xanthine is not decomposed. Urease is produced, but nitrate reductase is not produced. Acid is produced from D-galactose, D-glucose, glycerol, inositol, D-mannitol, D-mannose, trehalose, and citrate, but not from ribitol, L-arabinose, cellobiose, erythritol, lactose, maltose, melibiose, melezitose, raffinose, L-rhamnose, D-glucitol, and D-xylose.

The G+C content of the DNA is 67 to 68 mol%, as determined by the capillary electrophoresis procedure (19).

The cell wall contains *meso*-diaminopimelic acid, arabinose, and galactose. Nocardomycolic acids are present. As determined by HPLC, the mycolic acid patterns include two early peaks that are not found in any *N. brasiliensis* sensu stricto pattern. Contains predominant menaquinones similar to those in *N. asteroides* ATCC 19247^T as determined by an HPLC method (24).

The type strain is strain ATCC 51512 (= CIP 104600). This organism was isolated from a leg abscess on a patient suffering from ulcerative colitis. The G+C content of its DNA is 67.1 mol%.

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