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A Comparison of *Nocardia asteroides* and *Nocardia brasiliensis*

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SUMMARY: A description of *Nocardia asteroides* (Eppinger) Blanchard, based on the examination of 98 strains, is presented and compared with a description of *N. brasiliensis* (Lindenberg) Castellani and Chalmers, based on 50 strains. Characteristics common to freshly isolated strains, variants of the same strain, and old stock strains are used in the delineation of each species.

A taxonomic study of three closely related genera, *Mycobacterium*, *Nocardia* and *Streptomyces*, was begun in 1951 to find a group of stable correlating criteria for the identification of each taxon represented. An attempt was made to define each species in such a way that strains belonging to it could be recognized regardless of their stage of growth or history of cultivation *in vitro*. To effect this, newly isolated strains, variants of the same strain, and strains maintained *in vitro* for 1 to 30 or more years have been examined. Correlating characteristics common to all these strains have been sought for the delineation of the species. This work has been based on a collection now numbering 1254 strains. The collection has not been static, as strains from a variety of sources have been added continually. Newly received strains were examined as soon as possible for observation not only of the reactions of freshly isolated strains, but also of the variations of the older strains due to different conditions of maintenance used by the donors.

Nocardia asteroides (Eppinger) Blanchard, one of the species believed to be represented sufficiently in this collection, was described by Gordon & Mihm (1957). Since the 1957 report, several more strains of this species were found among those obtained from other investigators and are listed here.

Another aggregate of strains, possessing a distinctive group of characteristics, was considered to typify adequately the species *Nocardia brasiliensis* (Lindenberg) Castellani and Chalmers. The strains assigned to this species are described here and compared with those of *N. asteroides*.

METHODS

The observations and tests used in this study were described by Gordon & Smith (1955) and Gordon & Mihm (1957).

Strains used. Nineteen more recently acquired strains of *Nocardia asteroides* are listed in Table 1. At the time of their examination, seven of the 19 were recently isolated strains. These 19 strains bring the total of strains representing the species in this collection to 98. *Nocardia brasiliensis* is represented by the

50 strains listed in Table 3. Ten of these were examined within 6 months of isolation, and a few came from diagnostic laboratories in this country.

Table 1. *Additional strains identified as Nocardia asteroides (Eppinger) Blanchard*

Laboratory no.	Name when received, source, and strain name or number
747	<i>Nocardia asteroides</i> (Eppinger) Blanchard; Shirley McMillen, Hektoen Inst. for Medical Research; A. González Ochoa (652)
785	<i>N. asteroides</i> ; B. W. Lacey, Westminster School of Medicine (A ₁₀ , lung)
796, 797	<i>N. asteroides</i> ; F. C. Kull, Ciba Pharmaceutical Products Inc., Summit, N. J. (282, 341)
842	<i>N. asteroides</i> ; J. D. Schneidau, Jun., Tulane Univ. (314); ATCC (9970); C. W. Emmons (9956)
850	<i>N. brasiliensis</i> (Lindenberg) Castellani and Chalmers; J. D. Schneidau, Jun. (506); C. Lacaz (787)
783	<i>N. caprae</i> (Silberschmidt) Waksman and Henrici; B. W. Lacey (A ₂); NCTC (659)
722	<i>Nocardia</i> sp.; H. A. Waisman, Univ. of Wisconsin (pulmonary nocardiosis)
727, 728, 730	<i>Nocardia</i> spp.; L. Ajello, Communicable Disease Center, Chamblee, Ga. (M 170-6, M 171-6, M 173-6); W. M. Bowman
739	<i>Nocardia</i> sp.; W. K. Harris, Univ. of Mass. (44, bovine mastitis)
782	<i>Nocardia</i> sp.; B. W. Lacey (A ₁ , ulcerating cervical lymph node)
833, 834	<i>Nocardia</i> spp.; Mary P. Lechevalier, Rutgers Univ. (R 17ly, soil; AM 93, soil)
837	<i>Nocardia</i> sp.; J. B. Davis, Magnolia Petroleum Co., Dallas, Texas (107-236, utilizes ethane, Davis, Chase & Raymond, 1956)
572	<i>Proactinomyces asteroides</i> var. <i>crateriforme</i> Baldacci; N. M. McClung, Univ. of Kansas (10); Centraalbur. v. Schimmelcultures
709	<i>Streptomyces</i> sp.; S. Froman, Olive View Sanatorium, Calif. (24279, sputum)
830	Unidentified isolation; Charlotte C. Campbell, Walter Reed Army Medical Center (294, drainage from rectal fistula)

RESULTS

Nocardia asteroides (Eppinger) Blanchard

When received, none of the 19 strains had a specific name not initially borne by one or more of the strains first studied (Gordon & Mihm, 1957, Table 2); a discussion of their nomenclature need not be repeated.

Microscopic appearance. Cultures of 10 of the 19 strains grown on glycerol agar for 5 days at 28° showed branching filaments of varying lengths; seven, a mixture of rods and short or long filaments; one, coccoid forms, rods, and short branching filaments; and one, only thin, curved, lightly staining rods, often containing one or two dark granules. Seven of the 19 strains were not acid-fast. The remaining 12 varied from those which showed an occasional acid-fast rod or filament to those in which 50 % of organisms retained the carbol fuchsin. In some cultures, only portions of the filaments were acid-fast.

Eighteen of the 19 cultures formed loosely filamentous colonies, usually with extensive intertwining substrate (vegetative) hyphae. The colonies of the remaining culture were dense with a halo of short substrate hyphae. Under

Table 2. *Comparison of physiological characteristics*

Property	98 strains of <i>Nocardia</i> <i>asteroides</i> (% positive strains)	50 strains of <i>Nocardia</i> <i>brasiliensis</i> (% positive strains)
Decomposition of		
Casein	0	98
Gelatin	36	100
Tyrosine	0	100
Xanthine	0	0
Hydrolysis of starch	58	56
Acid from		
Adonitol	0	0
Arabinose	0	0
Erythritol	6	0
Galactose	24	92
Glucose	100	96
Glycerol	98	98
Inositol	2	100
Lactose	0	0
Maltose	6	0
Mannitol	0	94
Mannose	19	68
α -Methyl-D-glucoside	0	0
Raffinose	0	0
Rhamnose	33	0
Sorbitol	0	0
Xylose	0	0
Nitrite from nitrate	85	92
Growth at		
50°	24	0
45°	31	2
40°	90	56
37°	98	100
35°	100	100
28°	100	100
10°	12	30
Utilization of		
Acetate	100	100
Benzoate	0	0
Citrate	33	98
Malate	100	100
Propionate	100	100
Pyruvate	100	100
Succinate	100	98

the conditions of the observation of colonial morphology, the substrate hyphae of only four of the 19 strains fragmented into shorter forms. Aerial hyphae were seen on the colonies of all 19 strains, varying from rudimentary to long and branching. Those of some cultures coalesced, while those of others formed many short side branches, often in clusters. The aerial hyphae of two strains segmented into chains of even bead-like spores (Gordon & Mihm, 1958).

Macroscopic appearance. After 14 days of incubation on yeast glucose agar

at 28°, the growth of the 19 cultures varied from thin to abundant, restricted to spreading, flat, finely wrinkled, or warty and heaped-up, beige or peach shading to dark pink. Nine of the cultures were thickly covered with whitish aerial hyphae. The aerial hyphae of six cultures were sparse, while those of the remaining four were invisible to the unaided eye.

Table 3. *Strains identified as Nocardia brasiliensis (Lindenberg) Castellani and Chalmers*

Laboratory no.	Name when received, source, strain name or number
425, 426	<i>Actinomyces mexicanus</i> Boyd and Crutchfield; Isabel Christison, Duke Univ.; N. F. Conant (990, 993); A. González Ochoa (416, 413)
536	<i>Nocardia brasiliensis</i> (Lindenberg) Castellani and Chalmers; Isabel Christison; N. F. Conant (2393); M. da Silva (leg lesion)
556	<i>N. brasiliensis</i> ; E. N. Azarowicz, Univ. of Calif. (104); A. González Ochoa (468)
600-605, 684	<i>N. brasiliensis</i> ; A. González Ochoa, Inst. de Salubridad y Enfermedades Tropicales, Mexico (439, mycetoma of foot; 448, mycetoma of foot; 463, thoracic mycetoma; 456, mycetoma of knee; 473, thoracic mycetoma; 478, thoracic mycetoma; 414, facial mycetoma)
654-656, 679	<i>N. brasiliensis</i> ; E. N. Azarowicz (129, 127, 131, 128); Inst. Oswaldo Cruz (2587, 2589, 2588, 2592)
700-702	<i>N. brasiliensis</i> ; C. W. Emmons, Nat. Inst. of Health, Bethesda, Md. (9960, 9961, 9962); A. González Ochoa (mycetoma)
740	<i>N. brasiliensis</i> ; J. D. Schneidau, Jun., Tulane Univ. (382); A. González Ochoa
744, 790, 792	<i>N. brasiliensis</i> ; A. González Ochoa (409, 1002, 1008)
745, 746	<i>N. brasiliensis</i> ; Shirley McMillen, Hektoen Inst. for Medical Research; A. González Ochoa (410, 416)
748	<i>N. brasiliensis</i> ; J. E. Mackinnon, Inst. de Higiene, Montevideo, Uruguay (IHM 824); C. Lacaz
749, 750	<i>N. brasiliensis</i> ; J. E. Mackinnon (IHM 984, IHM, 1045); A. González Ochoa (thoracic mycetoma, Madura foot)
798-800	<i>N. brasiliensis</i> ; A. González Ochoa (4014, thoracic mycetoma; 4015, mycetoma of foot; 4016 ankle lesion)
844	<i>N. brasiliensis</i> ; J. D. Schneidau, Jun. (327); A. Batista (586)
845	<i>N. brasiliensis</i> ; J. D. Schneidau, Jun. (381); A. Batista (631); Inst. Pasteur, Paris (337)
846-849	<i>N. brasiliensis</i> ; J. D. Schneidau, Jun. (385, 386, 386 B, 505); C. Lacaz (95, 85, 85, 253)
851	<i>N. brasiliensis</i> ; J. D. Schneidau, Jun. (507, cutaneous lesion)
W 8485-W 8488	<i>N. mexicana</i> ; S. A. Waksman, Rutgers Univ.; A. González Ochoa (409, 413, 414, 415)
405	<i>N. mexicana</i> ; Julia M. Coffey, N.Y. State Dept. of Health (47296); J. B. Fischer; N. F. Conant
432	<i>N. mexicana</i> ; Isabel Christison; N. F. Conant (2178); A. González Ochoa
555	<i>N. mexicana</i> ; E. N. Azarowicz (73); Lucille K. Georg (149)
688	<i>N. mexicana</i> ; L. Ajello, Communicable Disease Center, Chamblee, Ga. (149); N. F. Conant (992)
N 2391	<i>N. pretoriana</i> Pijper and Pullinger; NCTC (2391)
731	<i>Nocardia</i> sp.; L. Ajello (M174-6); W. M. Bowman
733	Unidentified isolation; Charlotte C. Campbell, Walter Reed Army Medical Center (650)
774A, 774B	Unidentified isolations; Charlotte C. Campbell (variants of 8016, submandibular abscess)

Physiological characteristics. The physiological properties of the 19 more recently acquired strains were similar to those of the 79 previously studied strains of *Nocardia asteroides*. Characteristics of all the 98 strains are presented in Table 2. One of the two strains that did not grow at 37° was isolated from soil; the source of the second strain is unknown. Two of the nine strains that grew at 37° but not at 40° were obtained from human infections; five, from soil; and two were received without history of isolation. The following physiological criteria were considered the most important for the separation of strains of *N. asteroides* from those of other species of *Nocardia* and those received as streptomycetes: failure to attack casein, tyrosine, or xanthine; inability to form acid from adonitol, arabinose, erythritol, inositol, lactose, maltose, mannitol, α -methyl-D-glucoside, raffinose, sorbitol, or xylose; and non-utilization of benzoate. For the separation of strains of *N. asteroides* from senescent strains of all species of aerobic actinomycetes, which rarely give positive results to physiological tests, the following characteristics were used: acid production from glycerol and glucose, and the use of acetate, malate, propionate, pyruvate, and succinate as carbon sources. Variation by a strain from the pattern of these more dependable reactions in only one respect did not hinder its identification as *N. asteroides*.

Nocardia brasiliensis (Lindenberg) Castellani and Chalmers

The strains received as *Actinomyces mexicanus* Boyd & Crutchfield (1921), *Nocardia brasiliensis* (Lindenberg, 1909) Castellani & Chalmers (1913), and *N. pretoriana* Pijper & Pullinger (1927) were believed to agree with their original specific descriptions. The transfer of *A. mexicanus* and *N. pretoriana* to the synonymy of *N. brasiliensis* by González Ochoa (1945) and González Ochoa & Sandoval (1956) was confirmed. We were not able to find authority for the assignment of the species *mexicanus* of Boyd & Crutchfield (1921) to the genus *Nocardia*. Ota (1928), the authority usually cited, does not mention the species or the report of Boyd & Crutchfield. A search for effective publication of *N. mexicana*, therefore continues.

Microscopic appearance. After 5 days of growth at 28° on glycerol agar, 37 of the 50 cultures showed long branching filaments. The remaining 13 cultures consisted of coccobacilli, short to long rods, and short branching filaments. Nine of the cultures were not acid-fast, and the rest ranged from a few to 100 % acid-fast forms. Only portions of the filaments of approximately one-third of the cultures retained the carbol fuchsin.

All but two of the strains assigned to this species formed loosely or densely filamentous colonies or both. The substrate (vegetative) hyphae were usually long, rhizoid, intertwining, and were not observed to fragment. Two of the strains presented dense colonies with compact margins and densely filamentous colonies. All strains produced aerial hyphae that varied from rudimentary to short, straight or gnarled, or long and branching. The aerial hyphae of nearly half of the strains tended to coalesce. Round or irregular masses were scattered throughout the aerial hyphae of nine strains. Sporulation was not observed under the conditions of the examination. It is conceded,

however, that failure to produce spores might have been due to the use of media unsuitable for sporulation by these strains.

Macroscopic appearance. As with *Nocardia asteroides*, the appearance of the cultures on yeast glucose agar after 2 weeks of incubation at 28°, was one of the most mutable characteristics of the strains. The growth of some of the cultures was light yellow to yellowish orange, dry, spreading, finely wrinkled, and without aerial hyphae visible to the unaided eye. The growth of these strains might easily be mistaken for that of some mycobacteria. The growth of other cultures was beige to yellowish orange, spreading, thickly coated with whitish aerial hyphae, wrinkled, cracked, and identical in appearance with cultures received as *Streptomyces rimosus*. The remaining cultures ranged between the two extremes with beige, yellow, or yellowish orange, wrinkled, spreading growth. Some were sparsely covered with aerial hyphae, and a few formed amber to brown soluble pigment.

Physiological characteristics. The reactions of the 50 strains representing *Nocardia brasiliensis* are listed in Table 2 in comparison with those of *N. asteroides*. The following properties were deemed the most useful in the separation of strains of *N. brasiliensis* from those of *N. asteroides*: decomposition of casein and tyrosine and acid formation from inositol and mannitol. For the division of strains of *N. brasiliensis* from the strains of the remaining species of *Nocardia* and from strains received as streptomyces, the following criteria were the most important: failure to decompose xanthine; inability to produce acid from adonitol, arabinose, erythritol, lactose, maltose, α -methyl-D-glucoside, raffinose, rhamnose, sorbitol, or xylose; and non-utilization of benzoate. Acid formation from glucose and glycerol and the use of acetate, citrate, malate, propionate, pyruvate, and succinate as carbon sources provided positive characteristics in the pattern of reactions of this species. Deviation in one respect from this series of reactions was permitted in the identification of strains as *N. brasiliensis*.

DISCUSSION

The purpose of this study in seeking to identify old stock strains and variants as well as freshly isolated strains of each species is admittedly different from that of the diagnostic laboratory. Certain criteria which are reliable in the diagnostic laboratory, such as acid-fastness of freshly isolated strains of *Nocardia asteroides* and *N. brasiliensis*, must be accepted as variable when older strains are considered. Many of the tests and observations used here, on the other hand, require more time than is available to the diagnostician. In spite of the diversity in viewpoint and methods, the conclusions of this study confirm those of Almeida & Lacaz (1941), González Ochoa (1945), Moore, Lane & Gaul (1954), and others, in the recognition of *N. asteroides* and *N. brasiliensis* as two distinct species.

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