Some Criteria for the Recognition of *Nocardia madurae* (Vincent) Blanchard

By RUTH E. GORDON

*Institute of Microbiology, Rutgers, The State University,
New Brunswick, New Jersey, U.S.A.*

(Received 28 March 1966)

SUMMARY

A combination of characters for the description and identification of strains of *Nocardia madurae*, based on the examination of 44 strains, is presented. Although nearly all the 44 strains were received from medical laboratories, a few were isolated from soil. Some of the distinguishing properties of *N. madurae* are compared with those of *N. asteroides*, *N. caviae*, *N. brasiliensis*, *N. pelletieri* and *Streptomyces somaliensis*.

INTRODUCTION

During a comparative study of strains of mycobacteria, nocardias, and streptomycetes Gordon & Mihm (1962*a*) obtained as many strains as possible of *Nocardia asteroides* (Eppinger) Blanchard from the larger culture collections and collections of investigators interested in the species. Old stock strains, new isolates, and a number of their variants were examined. Properties common to all these strains were used to describe *N. asteroides* and to differentiate it from the other species represented in the collection which formed the basis of that study. As far as possible, this procedure was repeated in searching for criteria by which other species could be recognized.

Although a group of apparently useful properties for delineating *Nocardia madurae* (Vincent) Blanchard was found early in the study, progress in accumulating strains, particularly freshly isolated ones, to confirm the value of the distinguishing characteristics was very slow. Eventually enough strains of *N. madurae* were acquired from medical laboratories and from soil to provide reasonable assurance that the pattern of reactions presented here would be useful in the separation of *N. madurae* from other nocardias and from the streptomycetes. These reactions by which *N. madurae* can be recognized are compared with the reactions of *N. asteroides* (Eppinger) Blanchard, *N. brasiliensis* (Lindenberg) Castellani & Chalmers, *N. caviae* (Erikson) Gordon & Mihm, *N. pelletieri* (Laveran) Pinoy, and *Streptomyces somaliensis* (Brumpt) Waksman & Henrici. The patterns of reactions by which *N. caviae*, *N. pelletieri* and *S. somaliensis* are characterized, however, are based on very few strains and may be unreliable.

METHODS

Organisms. The strains listed in Tables 1, 3 and 4 and by Gordon & Mihm (1962*a*, *b*) were compared with each other and also with the other strains of mycobacteria, nocardias and streptomycetes in this collection.

Tests

The strains were examined according to the procedures described by Gordon (1966) with the following additions:

Hydrolysis of aesculin. The cultures were inoculated into aesculin broth (aesculin, 1 g.; ferric citrate, 0.5 g.; peptone, 10 g.; NaCl, 5 g.; water, 1000 ml.: Cowan & Steel, 1965), incubated at 28°, and observed for growth and blackening of the medium at 2, 4 and 6 weeks. A tube of the same broth without aesculin was also inoculated with each culture and used as a control.

Resistance to lysozyme. A solution of 0.1 g. lysozyme (Nutritional Biochemicals Corp., Cleveland, Ohio) in 60–65 ml. of sterile 0.01 N-HCl in a 100 ml. volumetric flask was boiled over an open flame for 20 min., and then plugged with sterile cotton-wool, cooled to room temperature, and brought to 100 ml. by the addition of sterile 0.01 N-HCl. Five ml. of the lysozyme solution was mixed with 95 ml. of sterile glycerol broth (peptone, 5 g.; beef extract, 3 g.; glycerol, 70 ml.; water, 1000 ml.; pH 7.0) and dispensed in 2.5 ml. amounts in sterile plugged tubes. A loopful of a 14- to 28-day culture in glucose or glycerol broth was inoculated into a tube of lysozyme broth and into a control tube of glycerol broth. The two tubes were observed for growth after 14 and 28 days of incubation at 28°.

Serial transfer in carbohydrate broth. Flasks of arabinose, mannitol, rhamnose and xylose broths were prepared by adding 10 ml. of a 10% (w/v) aqueous solution of each carbohydrate, sterilized by autoclaving separately, to 90 ml. of sterile broth containing (NH₄)₂HPO₄, 1 g.; KCl, 0.2 g.; MgSO₄·7H₂O, 0.2 g.; distilled water, 1000 ml. (Ayers, Rupp & Johnson, 1919). The broth was adjusted to pH 7.0 before the addition of 15 ml. of a 0.04% (w/v) solution of bromocresol purple. Each flask was heavily inoculated and incubated at 37°. After 2 or 3 months the remainder of the inoculum and the growth, if any, were pipetted to another flask of the same broth.

RESULTS

Nocardia madurae (Vincent) Blanchard

Microscopically, cultures on glycerol agar of the 44 strains of *Nocardia madurae* listed in Table 1 formed filaments which did not retain carbol fuchsin. The undisturbed colonies on Bennett's agar and on soil extract agar were densely or loosely filamentous. Under the conditions of the examination, 20 of the 44 strains produced aerial hyphae. The aerial hyphae of six of the 20 strains were long, with short curling side branches that segmented into short chains of spores; the aerial hyphae of the remaining 14 strains were sparse and varied from short and straight to long and branching.

After 2 weeks of incubation at 28° on Bennett's agar, the growth of some cultures was whitish, shiny, spreading, and either flat, coarsely wrinkled or folded. The whitish growth of some cultures contained a scattering of rose-coloured dots, and the crests of the folds of other whitish cultures were rose-coloured. The growth of other strains was beige, spreading, and flat or coarsely wrinkled; the growth of strains 780 and 1253 was reddish orange. Most of the strains did not form aerial hyphae visible to the unaided eye; the cultures of a few strains were, however, thickly coated with aerial hyphae; and others were sparsely coated. Brown, soluble

Table 1. *Strains identified as Nocardia madurae* (Vincent) Blanchard

Laboratory no.	Name when received, source, strain name or number
N 1070, N 5654	<i>Nocardia madurae</i> (Vincent) Blanchard; NCTC (1070, madura foot; 5654, Persia)
407	<i>N. madurae</i> ; Julia M. Coffey, N.Y. State Dept. of Health (47294); J. B. Fischer; N. F. Conant
431	<i>N. madurae</i> ; Isabel Christison, Duke Univ.; N. F. Conant (2177); A. González Ochoa (415)
507	<i>N. madurae</i> ; P. Thibault, Inst. Pasteur, Paris (Tunis)
554	<i>N. madurae</i> ; E. N. Azarowicz, Univ. of Calif. (62); C. Halde
614	<i>N. madurae</i> ; E. N. Azarowicz (26)
638	<i>N. madurae</i> ; A. González Ochoa, Inst. de Salubridad y Enfermedades Tropicales, Mexico (412, mycetoma of the foot)
635, 636, 791, 793	<i>N. madurae</i> ; A. González Ochoa (614, mycetoma of the foot; 420, mycetoma of the back; 485; 1004)
686	<i>N. madurae</i> ; L. Ajello, Communicable Disease Center, Chamblee, Ga. (A-528); J. Lamb (mycetoma)
706	<i>N. madurae</i> ; C. W. Emmons, Nat. Inst. of Health, Bethesda, Md. (9979); J. Lamb (mycetoma)
742	<i>N. madurae</i> ; J. D. Schneidau, Jun., Tulane Univ. (339, madura foot)
751-753	<i>N. madurae</i> ; J. E. Mackinnon, Inst. de Higue, Montevideo (IHM 769, Chile; IHM 869, North Africa; IHM 1154, India)
754	<i>N. madurae</i> ; J. E. Mackinnon (IHM 1390); A. González Ochoa (case 444, madura foot)
776, 777	<i>N. madurae</i> ; Jacqueline Walker, London School of Hygiene and Tropical Medicine (A. 260, A. 261; mycetoma pedis)
1189, 1190	<i>N. madurae</i> ; A. E. deArêa Leão, Inst. Oswaldo Cruz, Rio de Janeiro (1078, 2593)
1234	<i>N. madurae</i> ; G. Altmann, Tel Hashomer Government Hosp., Israel (TH 5, mycetoma pedis)
*	<i>N. madurae</i> ; Mary P. Lechevalier, Rutgers Univ. (P 56, Sal 1; soil)
780	<i>N. pelletieri</i> ; Jacqueline Walker (A. 292 (9972))
609	<i>N. pseudomadurae</i> ; E. N. Azarowicz (4)
W 3632 A	<i>Nocardia</i> sp.; G. Altmann, Military Hosp. No. 5, Israel (madura foot)
510	<i>Nocardia</i> sp.; P. Thibault (madura foot)
623	<i>Nocardia</i> sp.; E. N. Azarowicz (47)
637	<i>Nocardia</i> sp.; A. González Ochoa (486)
743	<i>Nocardia</i> sp.; Lucille K. Georg, Communicable Disease Center, Chamblee, Ga.; I. W. Kuhl (madura foot)
1136	<i>Nocardia</i> sp.; Joan M. Mihm, Rutgers Univ. (B-2, soil)
1210	<i>Nocardia</i> sp.; J. B. Fischer, Ontario Dept. of Health, Toronto (356, gluteal abscess)
571	<i>Proactinomyces pseudomadurae</i> Baldacci; N. M. McClung, Univ. of Kansas (3); Centraalbur. v. Schimmelcultures
1087, 1091	<i>Streptomyces albus</i> ; H. D. Tresner, Am. Cyanamid Co., Pearl River, N.Y. (AO-927-M-811, AO-928)
852	<i>S. madurae</i> (Vincent) González Ochoa & Sandoval; L. Briceño Iragorry, Univ. Central de Venezuela (FMC N. 107, Sablac; mycetoma of the foot)
972	<i>S. madurae</i> ; Mildred Feo, Univ. Central de Venezuela (FMC 96); D. Borelli (mycetoma of the foot)
971	<i>S. pelletieri</i> (Laveran) Waksman & Henrici; Mildred Feo (FMC 92); A. González Ochoa (thoracic mycetoma)
1092	<i>S. madurae</i> ; A. González Ochoa (4088, mycetoma of the foot)
1253, 1260	<i>S. madurae</i> ; F. Mariat, Inst. Pasteur, Paris (7087 B, 812)

* Two strains not added to this collection.

pigment was produced by some cultures, and a purple, soluble pigment by others. The appearance of the strains isolated from soil did not differ from that of strains from other sources. Because the growth of strains 571 and 609, received as *Nocardia*

pseudomaduræ and *Proactinomyces pseudomaduræ*, was whitish and beige, respectively, the pink and red pigments originally ascribed to them by Baldacci (1944) were presumably lost during cultivation *in vitro*.

The following combination of physiological properties (Table 2) was found to differentiate all 44 strains of *Nocardia maduræ* from the other strains in our

Table 2. *Comparison of physiological characteristics (% positive strains)*

Property	<i>Nocardia maduræ</i> (44 strains)	<i>Nocardia pelletieri</i> (15 strains)	<i>Streptomyces somaliensis</i> (22 strains)
Decomposition of			
Adenine	0	0	0
Casein	100	100	100
Hypoxanthine	100	100	0
Tyrosine	86	100	100
Urea	0	0	0
Xanthine	0	0	0
Growth at			
50°	0	7	0
45°	36	67	55
40°	98	100	91
35°	100	100	100
28°	100	100	100
10°	0	13	9
Survival at 50° for 8 hr	100	100	50
Nitrite from nitrate	100	100	0
Resistance to lysozyme	5	0	0
Utilization of			
Citrate	80	7	18
Malate	84	53	37
Succinate	80	0	45
Hydrolysis of			
Aesculin	100	0	0
Starch	100	13	41
Acid from			
Adonitol	91	0	0
Arabinose	100	0	0
Erythritol	0	0	0
Galactose	84	7	0
Glucose	100	100	50
Inositol	61	0	0
Lactose	55	0	0
Maltose	55	0	64
Mannitol	100	7	0
Mannose	89	0	5
Melibiose	0	0	0
α -m-D-glucoside	0	0	0
Raffinose	0	0	0
Rhamnose	100	0	0
Sorbitol	0	0	0
Trehalose	96	93	0
Xylose	100	0	0

collection: decomposed casein and hypoxanthine; did not attack adenine, urea or xanthine; grew at 40°; survived 50° for 8 hr; reduced nitrate to nitrite; were sensitive to lysozyme; hydrolysed aesculin and starch; acid formed from arabinose,

glucose, mannitol, rhamnose, trehalose and xylose; unable to produce acid from erythritol, melibiose, α -*m*-D-glucoside, raffinose or sorbitol. Utilization of citrate, malate and succinate; decomposition of tyrosine; and the formation of acid from adonitol, galactose and mannose were less dependable characters of *N. madurae*.

Table 3. *Strains identified as Nocardia pelletieri* (Laveran) Pinoy

Laboratory no.	Name when received, source, strain name or number
677	<i>Nocardia africana</i> Pijper & Pullinger; E. N. Azarowicz, Univ. of Calif. (152); Inst. Pasteur (84)
408	<i>N. pelletieri</i> (Laveran) Pinoy; Julia M. Coffey, N.Y. State Dept. of Health (47293)
513, 514	<i>N. pelletieri</i> ; P. Thibault, Inst. Pasteur, Paris
610	<i>N. pelletieri</i> ; E. N. Azarowicz, Univ. of Calif. (10)
687	<i>N. pelletieri</i> ; L. Ajello, Communicable Disease Center, Chamblee, Ga. (436); N.F. Conant (989)
705	<i>N. pelletieri</i> ; C. W. Emmons, Nat. Inst. of Health, Bethesda, Md. (9971); E. C. Smith (Naudi, mycetoma)
741	<i>N. pelletieri</i> ; J. D. Schneidau, Jun., Tulane Univ. (305); C. Lacaz (293)
755	<i>N. pelletieri</i> ; J. E. Mackinnon, Inst. de Higiene, Montevideo (IHM 614); Lab. de Parasitologie, Faculté de Médecine, Paris
756	<i>N. pelletieri</i> ; J. E. Mackinnon (IHM 615); A. Pijper (<i>Actinomyces africanus</i>)
757	<i>N. pelletieri</i> ; J. E. Mackinnon (IHM 1842, Abbot's)
778, 781	<i>N. pelletieri</i> ; Jacqueline Walker, London School of Hygiene and Tropical Medicine (A. 290, A. 293); E. C. Smith (mycetoma of the arm, mycetoma of the foot)
779	<i>N. pelletieri</i> ; Jacqueline Walker (A. 291, Merucci's)
N 4162	<i>Streptomyces pelletieri</i> (Laveran) González Ochoa & Sandoval; NCTC (4162); J. T. Duncan; E. C. Smith (Naudi)

Nocardia pelletieri (Laveran) Pinoy

Cultures on glycerol agar of the 15 strains of *Nocardia pelletieri* in our collection (Table 3) were filamentous and not acid-fast. Their colonies on soil extract agar and Bennett's agar were loosely or densely filamentous and did not exhibit aerial hyphae. One strain (757) on tyrosine and other agars, however, showed irregularly branching aerial hyphae.

Macroscopically the cultures after 2 weeks of incubation on Bennett's agar were pink, bright red, or dark red with a brownish, almost metallic tinge. Some were flat or coarsely wrinkled and spreading; others resembled a scattering of red-stained bread crumbs on the surface of the slant; and some formed a reddish brown soluble pigment.

During the cultivation of stock cultures of *Nocardia pelletieri* on Waksman's 'yeast dextrose' agar, beige-coloured variants developed spontaneously from strains 755 and 779.

The following pattern of physiological reactions of the strains of *Nocardia pelletieri*, separated them from the other strains in this collection: disintegrated casein, hypoxanthine and tyrosine; did not decompose adenine, urea or xanthine; grew at 40°; survived at 50° for 8 hr; reduced nitrate to nitrite; were sensitive to lysozyme; did not hydrolyse aesculin; formed acid from glucose and trehalose; did not produce acid from the remaining carbohydrates listed in Table 2.

Several unsuccessful attempts were made to obtain variants of strains of *Nocardia pelletieri* capable of forming acid from arabinose, mannitol, rhamnose and xylose

and thus to resemble strains of *N. madurae*. In one attempt, cultures of *N. pelletieri* were transferred serially in arabinose, mannitol, rhamnose or xylose broths for 18 months. Although several of the resulting cultures lost their bright red pigment and became yellowish or beige in colour, none of them produced acid from any of the four carbohydrates.

Streptomyces somaliensis (Brumpt) Waksman & Henrici

Cultures on glycerol agar of the 22 strains of *Streptomyces somaliensis* listed in Table 4 were filamentous and not acid-fast. The colonies of the 22 strains on soil extract agar and Bennett's agar were loosely or densely filamentous. Straight aerial hyphae which varied in length and branching were formed sparsely or abundantly by 13 of the 22 strains. Sporulation was not observed.

Table 4. *Strains identified as Streptomyces somaliensis*
(Brumpt) Waksman & Henrici

Laboratory no.	Name when received, source, strain name or number
632	<i>Actinomyces somaliensis</i> (Brumpt) St John-Brooks; A. González Ochoa, Inst. de Salubridad y Enfermedades, Mexico, D. F. (1064); J. T. Duncan
N 3026	<i>Streptomyces pelletieri</i> (Laveran) Waksman & Henrici; NCTC (3026); J. T. Duncan; E. C. Smith (mycetoma)
719	<i>S. somaliensis</i> (Brumpt) Waksman & Henrici; G. Altmann, Tel Hashomer Hosp., Israel (TH/4, mycetoma pedis)
1267-1269	<i>S. somaliensis</i> ; F. Mariat, Inst. Pasteur, Paris (313, 314, 315); J. Walker (A. 311, A. 310, A. 312)
1270-1276	<i>S. somaliensis</i> ; F. Mariat (383, 386, 387, 702, 733, 755, 761)
1278-1282, 1298	<i>S. somaliensis</i> ; F. Mariat (764A, 764B, 395, 765, 754, 746)
1295	<i>S. somaliensis</i> ; J. E. Mackinnon, Inst. de Higiene, Montevideo (IHM 1290)
1296, 1297	<i>S. somaliensis</i> ; J. E. Mackinnon (1531, 1532); I. G. Murray (1071, 1070)

The macroscopic appearance of the cultures of *Streptomyces somaliensis* grown on Bennett's agar for 2 weeks was not uniform. The growth was flat, finely or coarsely wrinkled, or warty; pigments were whitish, yellowish, beige, beige with a scattering of dark brown dots on the surface, or cocoa brown. Only four cultures had a coating of whitish aerial hyphae visible to the unaided eye; a few cultures formed a brown soluble pigment.

Physiological characteristics for recognizing the 22 strains of *Streptomyces somaliensis* (Table 2) were the clearing of casein and tyrosine; inability to decompose adenine, hypoxanthine, urea or xanthine; growth at 40°; failure to reduce nitrate to nitrite or to hydrolyse aesculin; sensitivity to lysozyme; and, with the exception of glucose and maltose, lack of acid production from carbohydrates. Eleven of the 22 strains formed acid from glucose, and 14 strains formed acid from maltose. Among the properties listed in Table 2, only decomposition of hypoxanthine, reduction of nitrate to nitrite, and acid formation from trehalose differentiated the strains of *Nocardia pelletieri* from those of *S. somaliensis*.

Comparison of six species

The more useful properties for recognizing *Nocardia madurae*, *N. pelletieri* and *Streptomyces somaliensis*, presented in Table 2, are compared with those of *N. asteroides*, *N. caviae* and *N. brasiliensis* (Gordon & Mihm, 1962a, b) in Table 5.

Although acid-fastness was a variable character of *N. asteroides*, *N. caviae* and *N. brasiliensis*, acid-fastness, when observed, was most helpful in identifying strains of these three species. Resistance to lysozyme was a reliable property of *N. asteroides*, *N. caviae*, *N. brasiliensis* and also of *S. lavendulae* and *S. rimosus*. Strains of *N. madurae*, *N. pelletieri*, *S. somaliensis*, *S. griseus* and *S. fradiae* were sensitive to lysozyme. Strains of *N. asteroides*, *N. caviae* and *N. brasiliensis* were also separated from those of *N. madurae*, *N. pelletieri* and *S. somaliensis* by decomposition of urea, and from *N. pelletieri* and *S. somaliensis* by hydrolysis of aesculin.

Table 5. *Some criteria for identifying six species of Nocardia and Streptomyces*

Property	% Positive strains.					
	<i>Nocardia asteroides</i> (142 strains)	<i>Nocardia caviae</i> (21 strains)	<i>Nocardia brasiliensis</i> (62 strains)	<i>Nocardia madurae</i> (44 strains)	<i>Nocardia pelletieri</i> (15 strains)	<i>Streptomyces somaliensis</i> (22 strains)
Acid-fastness	51	76	80	0	0	0
Decomposition of						
Casein	0	0	98	100	100	100
Hypoxanthine	4	100	94	100	100	0
Tyrosine	2	0	100	86	100	100
Urea	97	100	100	0	0	0
Xanthine	0	100	0	0	0	0
Survival at 50° for 8 hr	93	86	0	100	100	50
Nitrite from nitrate	88	100	90	100	100	0
Resistance to lysozyme*	99	100	100	5	0	0
Hydrolysis of aesculin	100†	100	100†	100	0	0
Acid from						
Adonitol	0	0	0	91	0	0
Arabinose	0	0	0	100	0	0
Glucose	97	100	97	100	100	50
Inositol	3	100	100	61	0	0
Mannitol	1	90	94	100	7	0
Rhamnose	32	5	0	100	0	0
Trehalose	—	—	—	96	93	0
Xylose	0	5	0	100	0	0

* 21 strains of *Streptomyces lavendulae* were resistant to lysozyme; 29 of 80 strains of *S. rimosus* were resistant; 23 strains of *S. fradiae* and 25 strains of *S. griseus* were not resistant.

† 50 strains examined.

Under the conditions of examination, the 142 strains of *Nocardia asteroides* in this collection (Table 5) were differentiated from *N. caviae* and *N. brasiliensis* by their inability to dissolve casein, hypoxanthine, tyrosine or xanthine; survival at 50° for 8 hr, and failure to form acid from inositol or mannitol. Although the strains of *N. caviae* resembled the strains of *N. asteroides* in many respects, *N. caviae* differed from *N. asteroides* in decomposing hypoxanthine and xanthine and in the production of acid from inositol and mannitol. The strains of *N. brasiliensis* attacked casein, hypoxanthine and tyrosine but not the crystals of xanthine; did not survive 50° for 8 hr; and formed acid from inositol and mannitol.

DISCUSSION

Nocardia madurae, *N. pelletieri* and *Streptomyces somaliensis* have been accepted as separate species by many investigators, and the distinctiveness of the three aggregates has been demonstrated by different workers in different ways. Disagreement on certain properties of the strains due to the use of different media and methods has occurred, but in my opinion this disagreement is relatively unimportant, because media and procedures are constantly changing. If today's workers recognize a taxonomic unit by different means, the investigators of the future will also recognize the same unit by procedures as yet unknown.

Confirmation is offered here (Table 3) for the assignment of *Nocardia africana* Pijper & Pullinger (1927) to the synonymy of *N. pelletieri* by González Ochoa & Vázquez Hoyos (1953), Conant *et al.* (1954), Mackinnon & Artagaveytia-Allende (1956) and Mariat (1958). An assignment of *Proactinomyces pseudomadurae* Baldacci (1944) to the synonymy of *N. madurae* (Table 1) by other workers was not found, although Mariat (1962) recognized rose- and red-pigmented strains from southern Europe and northern Africa as *N. madurae*.

While investigators apparently agree on the specific status of *Nocardia madurae*, *N. pelletieri* and *Streptomyces somaliensis*, the generic location of these three species is still unsettled. Waksman & Henrici (1948) transferred *N. pelletieri* (Laveran, 1906) Pinoy (1912) and *N. somaliensis* (Brumpt, 1906) Chalmers & Christopherson (1916) to the genus *Streptomyces*, and González Ochoa & Sandoval (1955) transferred *N. madurae* (Vincent, 1894) Blanchard (1896) to *Streptomyces*. These assignments, based on morphology, were accepted by Mackinnon & Artagaveytia-Allende (1956), Mariat (1958, 1962), Lynch & Moghraby (1961), Emmons, Binford & Utz (1963), and others.

Study of a goodly number of strains of nocardias and streptomycetes from a variety of sources revealed, however, that under the conditions of examination the characteristics of acid-fastness, fragmentation of the substrate hyphae, abundance of aerial hyphae, and segmentation of the aerial hyphae into chains of spores, used for the separation of the genera *Nocardia* and *Streptomyces*, were variable for *N. asteroides*; and that the properties of fragmentation of the substrate hyphae and formation of aerial hyphae and spores were variable for the streptomycetes (Gordon & Mihm, 1962*a*). Physiological criteria to separate the two genera were not found. The observation by Sohler, Romano & Nickerson (1958) that resistance to lysozyme could not be used for the separation of nocardias from streptomycetes, a retraction of an earlier report by Romano & Sohler (1956), is confirmed here (Table 5).

A division of the genera *Nocardia* and *Streptomyces* based on the chemical composition of the cell wall or of whole cells was offered by Romano & Nickerson (1956), Sohler, Romano & Nickerson (1958), Cummins & Harris (1958), Becker, Lechevalier, Gordon & Lechevalier (1964), Becker, Lechevalier & Lechevalier (1965), Yamaguchi (1965), and Murray & Proctor (1965). These workers demonstrated the presence of *meso*-diaminopimelic acid and arabinose in the cell-walls or in the whole cells of the nocardias and *LL*-diaminopimelic acid without arabinose in the streptomycetes. Of the strains listed in Tables 1, 3 and 4, 10 of *N. madurae*, three of *N. pelletieri*, and two of *S. somaliensis* were among those examined by Becker *et al.*

(1964). The remaining strains of *S. somaliensis* were also found to possess LL-diaminopimelic acid (H. A. & M. P. Lechevalier, unpublished data). The resulting generic separation was adopted in this report, but not wholeheartedly.

The statement of Cowan & Steel (1965) that 'The different kinds of bacteria are not separated by sharp divisions but by slight and subtle differences in characters so that they seem to blend into each other and resemble a spectrum', applies to the nocardias and streptomycetes. Morphologically and physiologically, various species of *Nocardia* and *Streptomyces* seem to form a spectrum, with *N. asteroides* at one end and *N. brasiliensis*, *N. madurae*, *N. pelletieri*, *S. somaliensis*, *S. lavendulae* and *S. griseus* following in that order. Any generic line dividing this spectrum will seem somewhat artificial and arbitrary, because it will assign related species to different genera.

The hierarchial system, the basis of our communication, is not well suited to the naming of the taxonomic units comprising a spectrum. As knowledge of an increasing number of strains and of an increasing number of their characters accumulates, dissatisfaction with the assignment of each strain to a genus and species may become generally widespread and result in a different, internationally adopted, framework of communication (Cowan, 1965). For the present, however, the need of communication dictates the assignment of a generic and a specific name to each taxonomic unit. Currently the *meso*- or LL-form of diaminopimelic acid and the presence or absence of arabinose in the cells or cell walls appear to be more stable properties than the morphological characteristics of these strains and therefore have been used here for generic separation. Future investigations, perhaps like those of Lanéele, Asselineau & Castelnuovo (1965) on the lipids of the cells may provide other, more acceptable, generic demarcation in the spectrum of the nocardias and streptomycetes.

This study was supported in part by research grant GB-2579 from the National Science Foundation, Washington, D.C., U.S.A. The author gratefully acknowledges this assistance and that of Dr and Mrs H. A. Lechevalier, who made the chromatographic examination of many of the strains, and of the investigators who kindly provided strains for this study.

REFERENCES

- AYERS, S. H., RUPP, P. & JOHNSON, JUN., W. T. (1919). A study of the alkali-forming bacteria found in milk. *Bull. U.S. Dept. Agric.* no. 782.
- BALDACCI, E. (1944). Contributo alla sistematica degli Attinomyceti. *Atti Ist. bot. Univ. Lab. crittogam Pavia* 3, 160.
- BECKER, B., LECHEVALIER, M. P. & LECHEVALIER, H. A. (1965). Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Appl. Microbiol.* 13, 236.
- BECKER, B., LECHEVALIER, M. P., GORDON, R. E. & LECHEVALIER, H. A. (1964). Rapid differentiation between *Nocardia* and *Streptomyces* by paper chromatography of whole-cell-hydrolysates. *Appl. Microbiol.* 12, 421.
- BLANCHARD, R. (1896). *Traité de Pathologie Générale*. Ed. by Ch. Bouchard, vol. 2, p. 811. Paris: G. Masson.
- BRUMPT, E. (1906). Les mycétomes. *Arch. Parasit. Paris* 10, 489.
- CHALMERS, A. J. & CHRISTOPHERSON, J. B. (1916). A Sudanese actinomycosis. *Ann. trop. Med. Parasit.* 10, 223.
- CONANT, N. F., SMITH, D. T., BAKER, R. D., CALLAWAY, J. L. & MARTIN, D. S. (1954). *Manual of Clinical Mycology*, 2nd ed. Philadelphia: W. B. Saunders.

- COWAN, S. T. (1965). Development of coding schemes for microbial taxonomy. In *Advances in Applied Microbiology*. Ed. by W. W. Umbreit, vol. 7. New York: Academic Press.
- COWAN, S. T. & STEEL, K. J. (1965). *Manual for the Identification of Medical Bacteria*. Cambridge University Press.
- CUMMINS, C. S. & HARRIS, H. (1958). Studies on the cell-wall composition and taxonomy of Actinomycetales and related groups. *J. gen. Microbiol.* **18**, 173.
- EMMONS, C. W., BINFORD, C. H. & UTZ, J. P. (1963). *Medical Mycology*. Philadelphia: Lea and Febiger.
- GONZÁLEZ OCHOA, A. & SANDOVAL, M. A. (1955). Características de los actinomicetes patógenos mas comunes. *Revta Inst. Salubr. Enferm. trop., Méx.* **16**, 149.
- GONZÁLEZ OCHOA, A. & VÁZQUEZ HOYOS, A. (1953). Relaciones serológicas de los principales actinomicetes patógenos. *Revta Inst. Salubr. Enferm. trop., Méx.* **13**, 177.
- GORDON, R. E. (1966). The taxonomy of soil bacteria. In *The Ecology of Soil Bacteria*. Ed. by T. R. G. Gray and B. Parkinson. Liverpool University Press. (In the Press.)
- GORDON, R. E. & MIHM, J. M. (1962*a*). The type species of the genus *Nocardia*. *J. gen. Microbiol.* **27**, 1.
- GORDON, R. E. & MIHM, J. M. (1962*b*). Identification of *Nocardia caviae* (Erikson) nov. comb. *Ann. N.Y. Acad. Sci.* **98**, 628.
- LANÉEEL, M.-A., ASSELINEAU, J. & CASTELNUOVO, G. (1965). Études sur les mycobactéries et les nocardiae. IV. Compositions des lipides de *Mycobacterium rhodochrous*, *M. pellegrino* sp., et de quelques souches de nocardiae. *Annls Inst. Pasteur, Paris* **108**, 69.
- LAVERAN, M. (1906). Tumeur provoquée par un microcoque rose en zooglées. *C.r. Séanc. Soc. Biol.* **61**, 340.
- LYNCH, J. B. & MOGHRABY, I. (1961). Mycetoma in the Sudan associated with *Streptomyces madurae*. *Trans. R. Soc. trop. Med. Hyg.* **55**, 446.
- MACKINNON, J. E. & ARTAGAVEYTIA-ALLENDE, R. C. (1956). The main species of pathogenic aerobic actinomycetes causing mycetomas. *Trans. R. Soc. trop. Med. Hyg.* **50**, 31.
- MARIAT, F. (1958). Physiologie des actinomycètes aérobies pathogènes. Recherches sur l'activité protéolytique et sur la nutrition azotée et carbonée de *Nocardia asteroides*, *N. brasiliensis*, *Streptomyces madurae*, *S. pelletieri* et *S. somaliensis*. *Mycopath. Mycol. appl.* **9**, 111.
- MARIAT, F. (1962). Critères de détermination des principales espèces d'Actinomycètes aérobies pathogènes. *Annls Soc. belge Méd. trop.* **4**, 651.
- MURRAY, I. G. & PROCTOR, A. G. J. (1965). Paper chromatography as an aid to the identification of *Nocardia* species. *J. gen. Microbiol.* **41**, 163.
- PIJPER, A. & PULLINGER, B. D. (1927). South African nocardiosis. *J. trop. Med. Hyg.* **30**, 153.
- PINOY, E. (1912). Discussion of paper by A. Thiroux & J. Pelletier. Mycétome à grains rouges de la paroi thoracique. Isolement et culture d'une nouvelle *Oospora* pathogène. *Bull. Soc. Path. exot.* **5**, 585.
- ROMANO, A. H. & NICKERSON, W. J. (1956). The biochemistry of the actinomycetales. Studies on the cell wall of *Streptomyces fradiae*. *J. Bact.* **72**, 478.
- ROMANO, A. H. & SOHLER, A. (1956). Biochemistry of the actinomycetales. II. A comparison of the cell wall composition of species of the genera *Streptomyces* and *Nocardia*. *J. Bact.* **72**, 865.
- SOHLER, A., ROMANO, A. H. & NICKERSON, W. J. (1958). Biochemistry of the actinomycetales. III. Cell wall composition and the action of lysozyme upon cells and cell walls of the actinomycetales. *J. Bact.* **75**, 283.
- VINCENT, M. H. (1894). Étude sur le parasite du 'Pied de Madura'. *Annls Inst. Pasteur, Paris* **8**, 129.
- WAKSMAN, S. A. & HENRICI, A. T. (1948). In *Bergey's Manual of Determinative Bacteriology*, 6th ed. Ed. by R. S. Breed, E. G. D. Murray and A. P. Hitchens, pp. 960, 965. Baltimore: Williams and Wilkins.
- YAMAGUCHI, T. (1965). Comparison of the cell-wall composition of morphologically distinct actinomycetes. *J. Bact.* **89**, 444.