Some Bits and Pieces of the Genus *Nocardia*: *N. carnea*, *N. vaccinii*, *N. transvalensis*, *N. orientalis* and *N. aerocolonigenes*

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(Received 22 May 1978)

Since 1951 when our taxonomic study of the genus *Nocardia* began, only eight strains of *N. carnea* (Rossi-Doria) Castellani & Chalmers, four strains of *N. vaccinii* Demaree & Smith, five of *N. transvalensis* Pijper & Pullinger, 21 of *N. orientalis* (Pittenger & Brigham) Pridham and 14 of *N. aerocolonigenes* (Shinobu & Kawato) Pridham have been found. These five groups of strains are described and are compared with strains of nine accepted species of *Nocardia*. With the possible exception of *N. orientalis*, more strains of these five taxa must be assembled and examined to establish the reliability of our descriptions and identifications.

INTRODUCTION

An ideal description of a microbial species is based on many strains, including freshly isolated strains, old stock strains and strains from different sources. In a collection of micro-organisms, however, some strains that appear to be taxonomically distinct accumulate only in very small numbers. Our collection of nocardiae, begun in 1951 as an outgrowth of several collections including the one initiated by S. A. Waksman in 1914, has its quota of these oddments. Because so few of these strains are available, descriptions of their species must remain tentative. These small aggregates of strains are, nevertheless, part of the genus *Nocardia*, and a few are described here and compared with strains of nine recognized species. We are well aware that when more strains can be examined some properties that now appear to be distinctive may be reduced to variable properties and that some of these aggregates of strains may prove to belong to currently accepted species.

METHODS

Strains. Our strains of the five aggregates are listed in Table 1 with their respective histories and, when known, their sources of isolation. Strains of nine species of *Nocardia*, included for comparative purposes, were representative strains of *N. amarae* and *N. hirsuta* (listed in Table 1) and the strains of seven other species used by Gordon *et al.* (1974).

Observations. Morphological, physiological and chromatographic examinations of the strains were made according to the methods described by Gordon *et al.* (1974) and Gordon & Barnett (1977). In addition, cellobiose, glycerol and melezitose were tested for acid formation. All cultures that did not produce acid from the various carbohydrates after 4 weeks incubation at 28 °C were incubated and re-examined at 8 weeks. For a representative number of strains of each of the 14 taxa, the method of Hecht & Causey (1976) was used for the determination of lipid characteristic of *Nocardia* spp., type A (LCN-A).

Table 1. *Designation and source of strains*

(a) Strains identified as <i>Nocardia carnea</i> (Rossi-Doria 1891) Castellani & Chalmers 1913	
ATCC 6847	<i>N. carnea</i> ; R. St. John-Brooks, NCTC (3527); V. Puntoni
IMRU 1341	<i>Streptomyces</i> sp.; J. W. Brandsburg, Natl Insts of Health, Bethesda, Md. (p2898, from pleural fluid)
IMRU 1406	<i>N. convaluta</i> ; M. Goodfellow, Univ. of Leicester (N95); A. Gonzalez Ochoa (98)
IMRU 1642	Unidentified isolation; L. E. Vázquez, Puerto Rico Medical Center, San Juan, Puerto Rico (Cm624, eye of a patient)
IMRU 1674	<i>Nocardia</i> sp.; K. P. Schaal, Univ. zu Köln (N14)
IMRU 1684	Unidentified isolation; J. B. Fischer, Ontario Ministry of Health, Toronto (FT55-75, from pleural fluid)
IMRU 3419	<i>N. carnea</i> ; ATCC 6847; NCTC 3527; V. Puntoni
IMRU 3809	<i>N. carnea</i> ; Lederle Laboratories, Pearl River, N.Y.; ATCC 6847
(b) Strains identified as <i>Nocardia vaccinii</i> Demaree & Smith 1952	
ATCC 11092*	<i>N. vaccinii</i> ; N. R. Smith, U.S. Dept of Agr. (BG19, from blueberry gall)
IMRU 1072, 1074	<i>Streptomyces</i> spp.; J. B. Routien, Chas. Pfizer & Co., Brooklyn, N.Y. (AR368, 38366-20 and AP9545, 50392-017)
IMRU 3500*	<i>N. vaccinii</i> ; J. B. Demaree, U.S. Dept of Agr. (BG19, from blueberry gall)
(c) Strains identified as <i>Nocardia transvalensis</i> Pijper & Pullinger 1927	
NCTC 2392*	<i>N. transvalensis</i> ; S. T. Cowan, NCTC (2392); A. Pijper (16)
IMRU 1222	Unidentified isolation; F. Mariat, Inst. Pasteur (945, from a mycetoma)
IMRU 1301	<i>N. brasiliensis</i> ; M. Moore, Homer G. Phillips Hospital, St Louis, Mo. (1, from a mycetoma)
IMRU 1583	<i>N. asteroides</i> ; M. Magnusson, Statens Seruminstitut, Copenhagen (667); F. Mariat (IP766 Cameroun, from a mycetoma)
IMRU 3426*	<i>N. transvalensis</i> ; ATCC 6865; NCTC 2392
(d) Strains identified as <i>Nocardia orientalis</i> (Pittenger & Brigham 1956) Pridham 1970	
ISP 5040*	<i>Streptomyces orientalis</i> ; International Streptomyces Project (5040); R. C. Pittenger (M43-05865)
IMRU 1107	<i>Streptomyces</i> sp.; J. M. Mihm, Rutgers Univ. (from rotting egg plant)
IMRU 1148, 1149	<i>Streptomyces</i> spp.; T. H. Stoudt, Merck & Co., Rahway, N.J. (c of 'Red' group and D of 'Red' group)
IMRU 1311	<i>Nocardia-Streptomyces</i> group; M. P. Lechevalier, Rutgers Univ. (F719, from a clinical specimen)
IMRU 1389, 1390, 1409	<i>N. brasiliensis</i> ; J. Scharfen, Inst. of Epidemiology and Microbiology, Prague (AK-16/65, w-113/66 and AK-4348)
IMRU 1591, 1592	<i>Micropolyspora</i> spp.; L. K. Georg, Center for Disease Control, Atlanta, Ga. (w2100 and w2101, from Sudan soil)
IMRU 1600	<i>N. orientalis</i> ; T. G. Pridham, U.S. Dept of Agr., Peoria, Ill. (NRRL B-3891)
IMRU 1601	<i>Micropolyspora</i> sp.; L. K. Georg, Center for Disease Control, Atlanta, Ga. (w2156, from Sudan soil)
IMRU 1627, 1628	<i>N. orientalis</i> ; T. G. Pridham, U.S. Dept of Agr., Peoria, Ill. (NRRL B-2451 and B-2452)
IMRU 1631-1633	<i>N. orientalis</i> ; C. E. Higgins, Eli Lilly & Co., Indianapolis, Ind. (A25284, A37317 and M48-E2570)
IMRU 1637, 1640	<i>Nocardia</i> spp.; M. P. Lechevalier, Rutgers Univ. (5-8, from soil containing 9400 p.p.m. Zn, and 11-14, from soil with low Zn content)
IMRU 1644	<i>N. orientalis</i> ; G. Carroll, Center for Disease Control, Atlanta, Ga. (w2684, from spinal fluid)
IMRU 1671	Unidentified isolation; M. P. Lechevalier, Rutgers Univ. (26-75, from soil)
(e) Strains identified as <i>Nocardia aerocolonigenes</i> (Shinobu & Kawato 1960) Pridham 1970	
ISP 5034*	<i>Streptomyces aerocolonigenes</i> ; International Streptomyces Project (5034); R. Shinobu (701)
IMRU 550	<i>Nocardia</i> sp.; E. N. Azarowicz, Univ. of California, Los Angeles (30, from California soil)
IMRU 983	<i>Nocardia</i> sp.; M. P. Lechevalier, Rutgers Univ. [1a(3), from soil]
IMRU 1106	<i>Streptomyces</i> sp.; J. M. Mihm, Rutgers Univ. (from soil)
IMRU 1432	<i>Nocardia</i> sp.; M. P. Lechevalier, Rutgers Univ. (r3)
IMRU 1546, 1566	Unidentified isolations; T. G. Pridham, U.S. Dept of Agr., Peoria, Ill. (NRRL B-3274, from desert soil, and B-11267, from garden soil)
IMRU 1589	<i>N. brasiliensis</i> ; L. K. Georg, Center for Disease Control, Atlanta, Ga. (w1894, from spinal fluid)
IMRU 1593, 1594, 1599	Unidentified isolations; L. K. Georg, Center for Disease Control, Atlanta, Ga. (w2104, w2105 and w2158, from Sudan soil)
IMRU 1663, 1667, 1668	Unidentified isolations; M. P. Lechevalier, Rutgers Univ. (26-39 and 26-67, from soil heavily polluted with Zn and Cd, and 26-69, from soil)

Table 1. *Designation and source of strains*—continued

(f) Strains examined of <i>Nocardia amarae</i> Lechevalier & Lechevalier 1974	
IMRU 1529–1533	<i>Nocardia amarae</i> ; M. P. Lechevalier, Rutgers Univ. (Se3, Se51, Se64, Se85 and Se87)
IMRU 1535–1539	<i>Nocardia amarae</i> ; M. P. Lechevalier, Rutgers Univ. (Se107, Se110, Se111, Se117 and Se102)
IMRU 1604–1606	Unidentified isolations; V. D'Aco, 12D Fletcher Rd, Monsey, N.Y. (9c, 10a and 10b, from activated sludge)
IMRU 3960*	<i>Nocardia amarae</i> ; M. P. Lechevalier, Rutgers Univ. (Se6)
(g) Strains examined of <i>Nocardia hirsuta</i> (<i>Saccharopolyspora hirsuta</i>) Lacey & Goodfellow 1975	
IMRU 1437–1439	<i>Nocardia</i> spp.; J. Lacey, Rothamsted Experimental Station, England (A153, A197 and A216)
IMRU 1461–1463	<i>Nocardia</i> spp.; J. Lacey, Rothamsted Experimental Station, England (A205, A206 and A770)
IMRU 1475, 1476	<i>Nocardia</i> spp.; J. Lacey, Rothamsted Experimental Station, England (A185 and A195)
IMRU 1547–1557	<i>Nocardia</i> spp.; J. Lacey, Rothamsted Experimental Station, England (A1132, A1133, A1134, A1135, A1136, A1137, A1138, A1139, A1140, A1141 and A1142)
IMRU 1558*–1562	<i>Nocardia</i> spp.; J. Lacey, Rothamsted Experimental Station, England (A1143, A1144, A1145, A1146 and A1147)

* Nomenclatural type strain.

ATCC, American Type Culture Collection; IMRU, Waksman Institute of Microbiology, Rutgers University; NCTC, National Collection of Type Cultures; ISP, International Streptomyces Project.

RESULTS

Nocardia carnea

Macroscopically the growth of cultures of *N. carnea* at 1 to 2 weeks on yeast dextrose agar was abundant, cream- or peach-coloured and moderately or thickly covered with whitish aerial hyphae. They closely resembled cultures of some strains of *N. asteroides*. Cultures of ATCC 6847, obtained from the NCTC in 1938, still formed an abundance of aerial hyphae. Microscopically the colonies were loosely filamentous, and the aerial hyphae of two of the eight cultures (ATCC 6847 and IMRU 3809) segmented into conidia. Smears of cultures grown on glycerol agar showed coccoid forms, rods and/or filaments. Cultures of two of the eight strains were acid-fast.

The physiological properties of strains of *N. carnea* were very like those of strains of *N. asteroides* (Tables 2 and 3). Positive characteristics of strains of *N. carnea* (positive for 85 to 100 % of the strains) were: resistance to lysozyme and rifampin; hydrolysis of aesculin; acid formation from glucose, glycerol, mannitol, sorbitol and trehalose; utilization of succinate; reduction of nitrate to nitrite; and survival at 50 °C for 8 h. Negative characteristics (positive for 0 to 14 % of the strains) were: decomposition of adenine, casein, hypoxanthine, tyrosine, urea and xanthine; hydrolysis of hippurate; production of acid from adonitol, arabinose, cellobiose, erythritol, lactose, maltose, melezitose, melibiose, methyl α -glucoside, raffinose, rhamnose and xylose; and utilization of benzoate, mucate and tartrate. Only the inability to form urease and the ability to produce acid from mannitol and sorbitol separated strains of *N. carnea* from typical strains of *N. asteroides*.

Nocardia vaccinii

On yeast dextrose agar, the growth of cultures of *N. vaccinii* was cream- or peach-coloured and moderately covered with white aerial hyphae. When examined microscopically, colonies of the four strains were loosely filamentous and showed short or long, branching aerial hyphae. Conidia were formed by the aerial hyphae of one strain (IMRU 1072). Cultures grown on glycerol agar were composed of partially acid-fast rods and filaments.

Positive physiological reactions of the strains were: decomposition of urea; resistance to lysozyme; hydrolysis of aesculin and starch; formation of acid from arabinose, glucose, glycerol and mannitol; utilization of citrate, mucate and succinate; reduction of nitrate to

Table 2. *Some physiological properties of nine species and five aggregates of Nocardia*

Results indicate the percentage frequency of positive characters.

Property	<i>N. asteroides</i> *(137)	<i>N. carnei</i> (8)	<i>N. vaccinii</i> (4)	<i>N. amarcae</i> (14)	<i>N. caviae</i> (43)	<i>N. brasiliensis</i> (72)	<i>N. transvalensis</i> (5)	<i>N. autotrophica</i> (31)	<i>N. hirsuta</i> (24)	<i>N. orientalis</i> (21)	<i>N. aerocolonigenes</i> (14)	<i>N. dassonvillei</i> (46)	<i>N. madurae</i> (47)	<i>N. pelletieri</i> (25)
Acid-fastness	61	25	100	0	65	78	80	0	0	0	0	0	0	0
Decomposition of:														
Adenine	0	0	0	0	2	6	40	97	100	0	0	100	0	0
Casein	0	0	0	0	2	93	0	0	100	100	100	98	98	100
Hypoxanthine	4	0	0	0	100	92	100	94	100	100	100	100	98	76
Tyrosine	1	0	0	0	14	99	100	97	100	95	100	100	91	100
Urea	96	0	100	100	95	99	100	94	100	95	93	39	0	0
Xanthine	0	0	0	0	100	0	40	77	100	67	0	100	0	0
Resistance to:														
Lysozyme	100	100	100	0	100	100	100	0	0	14	100	2	9	0
Rifampin	92	88	50	64	97	100	100	0	0	81	0	0	73	52
Hydrolysis of:														
Aesculin	100	100	100	100	100	100	100	90	100	100	100	20	98	0
Hippurate	20	13	0	7	21	6	0	23	83	71	57	100	4	0
Starch	67	38	100	100	56	64	100	100	100	95	100	100	100	0
Acid from:														
Adonitol	1	0	0	0	2	0	100	94	100	100	57	0	91	0
Arabinose	0	0	100	0	19	0	0	81	0	100	100	59	100	0
Cellobiose	0†	0	0	0	0	0	0	84	100	100	100	100	100	0
Erythritol	4	0	0	0	0	0	100	100	100	100	0	0	0	0
Glucose	97	100	100	100	100	99	100	100	100	100	100	100	100	100
Glycerol	95	100	100	100	100	100	100	100	100	100	100	100	100	0
Inositol	3	25	75	79	100	100	60	32	100	95	100	0	60	0
Lactose	0	0	0	0	0	0	0	95	100	100	100	0	55	0
Maltose	2	0	25	100	7	3	0	90	100	81	100	100	53	0
Mannitol	1	88	100	100	81	94	80	100	100	100	86	100	100	0
Mannose	18	38	0	100	35	65	20	100	100	100	100	93	94	0
Melezitose	0†	0	0	0	0	0	0	100	0	33	0	0	11	0
Melibiose	0	0	0	0	0	0	0	0	0	38	100	11	0	0
Methyl α -glucoside	0	0	0	0	0	0	0	42	100	90	14	30	0	0
Raffinose	0	0	0	0	0	0	0	95	24	71	0	0	0	0
Rhamnose	33	0	75	79	7	3	0	10	100	57	79	52	100	0
Sorbitol	0	100	25	0	2	0	80	94	100	38	7	0	0	0
Trehalose	60	88	50	100	55	96	80	90	100	81	100	76	96	96
Xylose	0	0	75	0	9	1	0	100	100	100	100	67	100	0
Utilization of:														
Benzoate	0	0	0	0	0	1	0	39	100	48	0	0	6	0
Citrate	33	25	100	0	42	99	100	97	100	100	100	98	83	0
Mucate	0	0	100	0	5	0	0	0	100	48	7	9	0	0
Succinate	95	88	100	100	100	100	100	100	100	100	100	100	83	0
Tartrate	0	0	0	0	0	0	0	23	100	5	0	9	0	0
Nitrite from nitrate	91	88	100	100	95	89	100	45	0	43	50	87	98	100
Survival at 50 °C, 8 h	96	100	100	0	95	7	100	68	100	81	21	96	100	100

* Numbers in parentheses indicate the number of strains examined.

† One hundred strains examined.

Table 3. *Some physiological properties of typical strains of 14 taxa of Nocardia*

Property	<i>N. asteroides</i>	<i>N. cavia</i>	<i>N. vacinii</i>	<i>N. amarae</i>	<i>N. caviae</i>	<i>N. brasiliensis</i>	<i>N. transvalensis</i>	<i>N. autotrophica</i>	<i>N. hirsuta</i>	<i>N. orientalis</i>	<i>N. aerocolonigenes</i>	<i>N. dassonvillei</i>	<i>N. madurae</i>	<i>N. pelletieri</i>
Acid-fastness	v	v	+	—	v	v	v	—	—	—	—	—	—	—
Decomposition of:														
Adenine	—	—	—	—	—	—	v	+	+	—	—	+	—	—
Casein	—	—	—	—	—	+	—	—	+	+	+	+	+	+
Hypoxanthine	—	—	—	—	+	+	+	+	+	+	+	+	+	v
Tyrosine	—	—	—	—	—	+	+	+	+	+	+	+	+	+
Urea	+	—	+	+	+	+	+	+	+	+	+	v	—	—
Xanthine	—	—	—	—	+	—	v	v	+	v	—	+	—	—
Resistance to:														
Lysozyme	+	+	+	—	+	+	+	—	—	—	+	—	—	—
Rifampin	+	+	v	v	+	+	+	—	—	v	—	—	v	v
Hydrolysis of:														
Aesculin	+	+	+	+	+	+	+	+	+	+	+	v	+	—
Hippurate	v	—	—	—	v	—	—	v	v	v	v	+	—	—
Starch	v	v	+	+	v	v	+	+	+	+	+	+	+	—
Acid from:														
Adonitol	—	—	—	—	—	—	+	+	+	+	v	—	+	—
Arabinose	—	—	+	—	v	—	—	v	—	+	+	v	+	—
Cellobiose	—	—	—	—	—	—	—	v	+	+	+	+	+	—
Erythritol	—	—	—	—	—	—	+	+	+	+	—	—	—	—
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	—
Inositol	—	v	v	v	+	+	v	v	+	+	+	—	v	—
Lactose	—	—	—	—	—	—	—	—	+	+	+	—	v	—
Maltose	—	—	v	+	—	—	—	+	+	v	+	+	v	—
Mannitol	—	+	+	+	v	+	v	+	+	+	+	+	+	—
Mannose	v	v	—	+	v	v	v	+	+	+	+	+	+	—
Melezitose	—	—	—	—	—	—	—	+	—	v	—	—	—	—
Melibiose	—	—	—	—	—	—	—	—	—	v	+	—	—	—
Methyl α -glucoside	—	—	—	—	—	—	—	v	+	+	—	v	—	—
Raffinose	—	—	—	—	—	—	—	—	+	v	v	—	—	—
Rhamnose	v	—	v	v	—	—	—	—	+	v	v	v	+	—
Sorbitol	—	+	v	—	—	—	v	+	+	v	—	—	—	—
Trehalose	v	+	v	+	v	+	v	+	+	v	+	v	+	+
Xylose	—	—	v	—	—	—	—	+	+	+	+	v	+	—
Utilization of:														
Benzoate	—	—	—	—	—	—	—	v	+	v	—	—	—	—
Citrate	v	v	+	—	v	+	+	+	+	+	+	+	v	—
Mucate	—	—	+	—	—	—	—	—	+	v	—	—	—	—
Succinate	+	+	+	+	+	+	+	+	+	+	+	+	v	—
Tartrate	—	—	—	—	—	—	—	v	+	—	—	—	—	—
Nitrite from nitrate	+	+	+	+	+	+	+	v	—	v	v	+	+	+
Survival at 50 °C, 8 h	+	+	+	—	+	—	+	v	+	v	v	+	+	+

+, 85 to 100 % of the strains positive; —, 0 to 14 % of the strains positive; v, 15 to 84 % of the strains positive. See Table 2 for numbers of strains examined.

nitrite; and survival at 50 °C for 8 h. The strains did not dissolve casein or the crystals of adenine, hypoxanthine, tyrosine or xanthine; hippurate was not hydrolysed; acid was not produced from adonitol, cellobiose, erythritol, lactose, mannose, melezitose, melibiose, methyl α -glucoside or raffinose; and benzoate and tartrate were not used as sources of carbon (Tables 2 and 3). Only three properties (production of acid from arabinose and mannitol and the utilization of mucate) divided the four strains of *N. vaccinii* from strains of *N. asteroides*. The presence of urease, production of acid from arabinose and use of mucate distinguished strains of *N. vaccinii* from strains of *N. carnea*. Strains of *N. vaccinii* could be differentiated from strains of *N. amarae* by their acid-fastness, formation of acid from arabinose, failure to produce acid from mannose, resistance to lysozyme, utilization of citrate and mucate, and survival at 50 °C for 8 h.

Nocardia transvalensis

The growth of cultures of *N. transvalensis* on yeast dextrose agar was abundant and thickly coated with whitish aerial hyphae. The pigments of the growth were cream or pale purple. The colonies of the five strains were loosely filamentous, and their aerial hyphae were long and branching. Conidia were not observed. The rods and filaments of four of the five strains grown on glycerol agar were 10 to 50 % acid-fast. A culture of the fifth strain (IMRU 1583) exhibited only non-acid-fast coccoid bodies.

The distinctive pattern of characteristics of our five strains of *N. transvalensis* consisted of positive reactions on hypoxanthine, tyrosine, urea, lysozyme, rifampin, aesculin, starch, adonitol, erythritol, glucose, glycerol, citrate, succinate, nitrate and survival at 50 °C for 8 h. Reactions were negative on casein, arabinose, cellobiose, lactose, maltose, melezitose, melibiose, methyl α -glucoside, raffinose, rhamnose, xylose, hippurate, benzoate, mucate and tartrate (Tables 2 and 3). This pattern of reactions differentiated strains of *N. transvalensis* from typical strains of (i) *N. caviae* by their positive reactions on tyrosine, adonitol and erythritol, (ii) *N. brasiliensis* by their inability to decompose casein and by their ability to form acid from adonitol and erythritol and to survive at 50 °C for 8 h and (iii) *N. autotrophica* by their resistance to lysozyme and rifampin and their negative reactions on cellobiose, maltose, melezitose and xylose.

Nocardia orientalis

Cultures of *N. orientalis* grew well on yeast dextrose agar. The growth, often wrinkled, was cream or peach in colour and sparsely or moderately covered with aerial hyphae. Microscopically colonies of the 21 strains were loosely filamentous, and colonies of 20 of the 21 strains produced long, straight, branching aerial hyphae, while those of one strain were short. The aerial hyphae of 11 strains formed conidia. Acid-fastness was not observed in any of the cultures.

Typical strains of *N. orientalis* decomposed casein, hypoxanthine, tyrosine and urea; they hydrolysed aesculin and starch; they formed acid from adonitol, arabinose, cellobiose, erythritol, glucose, glycerol, inositol, lactose, mannitol, mannose, methyl α -glucoside and xylose; they utilized citrate and succinate; and survived at 50 °C for 8 h (Tables 2 and 3). The strains were unable to dissolve the crystals of adenine. They were sensitive to lysozyme and did not use tartrate as a carbon source.

Strains of *N. orientalis* could be distinguished from strains of (i) *N. autotrophica* by their inability to decompose adenine and their positive reactions on casein and lactose, (ii) *N. hirsuta* by their negative action on adenine and tartrate and their ability to produce acid from arabinose and (iii) *N. aerocolonigenes* by their positive action on erythritol and methyl α -glucoside and their sensitivity to lysozyme.

Nocardia aerocolonigenes

Although cultures of *N. aerocolonigenes* grew well on yeast dextrose agar, their viability was short compared with that of cultures of other *Nocardia* spp. The transfer of working stock cultures at intervals of no longer than 2 weeks is recommended as well as a substantial supply of lyophilized cultures.

The growth of the cultures on yeast dextrose agar was cream-coloured or pale orange. As reported by Shinobu & Kawato (1960), the cultures quickly lost their ability to form aerial hyphae upon continued transfer. When first observed microscopically, the loosely filamentous colonies of eight of the 14 strains formed short or long, straight, branching aerial hyphae, and only one (IMRU 550) produced conidia. The cultures grown on glycerol agar were not acid-fast.

Positive physiological reactions of 85 to 100 % of the strains were: decomposition of casein, hypoxanthine, tyrosine and urea; resistance to lysozyme; hydrolysis of aesculin and starch; production of acid from arabinose, cellobiose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, melibiose, trehalose and xylose; and utilization of citrate and succinate. Negative characteristics (positive for 0 to 14 % of the strains) were: decomposition of adenine and xanthine; resistance to rifampin; formation of acid from erythritol, melezitose, methyl α -glucoside and sorbitol; and utilization of benzoate, mucate and tartrate. Among these properties, decomposition of adenine and xanthine, production of acid from inositol, lactose and melibiose and resistance to lysozyme differentiated strains of *N. aerocolonigenes* from strains of *N. dassonvillei*. Strains of *N. aerocolonigenes* were distinguished from those of *N. madurae* by the presence of urease, production of acid from melibiose and resistance to lysozyme.

Chromatographic determinations

Whole-cell hydrolysates of representative strains of *N. carnea*, *N. vaccinii*, *N. transvalensis*, *N. orientalis* and *N. aerocolonigenes* were examined for the presence of *meso*-diaminopimelic acid (*meso*-DAP), arabinose, galactose, glucose, madurose, mannose and lipid characteristic of *Nocardia* spp., type A (LCN-A). The results were compared with those of representative strains of nine species of nocardiae (Table 4). Representative strains of each taxon included the nomenclatural type or neotype strain, if designated. Under the conditions of our test, 90 % of the strains of *N. asteroides*, 95 % of the strains of *N. caviae* and 100 % of those of the remaining 12 taxa contained *meso*-DAP. Hydrolysates of strains of *N. carnea* and *N. transvalensis* contained arabinose, galactose, glucose, mannose and LCN-A, but not madurose. Strains of *N. vaccinii* were positive for arabinose, galactose, glucose and LCN-A. Two of the four strains contained mannose, but none of them had madurose. In having arabinose, galactose and LCN-A without madurose, these three aggregates (*N. carnea*, *N. vaccinii*, and *N. transvalensis*) chromatographically resembled strains of *N. asteroides*, *N. amarae*, *N. caviae* and *N. brasiliensis*.

Although our 21 strains of *N. orientalis* also contained arabinose and galactose but no madurose, hydrolysates of the 18 strains examined did not have LCN-A and thus resembled strains of *N. autotrophica* and *N. hirsuta*. The 14 strains of *N. aerocolonigenes* contained galactose and mannose; seven of the strains had glucose; four had madurose; and none had arabinose or LCN-A. In their lack of arabinose and LCN-A, the strains of *N. aerocolonigenes* resembled strains of *N. dassonvillei*, *N. madurae* and *N. pelletieri*. The presence of madurose in four of the 14 strains also indicated a relationship between *N. aerocolonigenes* and *N. madurae* and *N. pelletieri*.

Table 4. Presence of diaminopimelic acid, sugars and lipid characteristic of *Nocardia* species, type A (LCN-A) in whole-cell hydrolysates of cultures of *Nocardia* species and aggregates

Species	No. of strains examined	Percentage of strains containing:						LCN-A*
		<i>meso</i> -DAP	Arabinose	Galactose	Glucose	Madurose	Mannose	
<i>N. asteroides</i>	39	90	100	100	85	0	87	100 (7)
<i>N. carneae</i>	8	100	100	88	88	0	100	100 (5)
<i>N. vaccinii</i>	4	100	100	100	100	0	50	100 (4)
<i>N. amarae</i>	8	100	100	13	50	0	0	100 (6)
<i>N. caviae</i>	22	95	100	100	68	0	45	100 (8)
<i>N. brasiliensis</i>	17	100	100	100	94	0	88	100 (7)
<i>N. transvalensis</i>	5	100	100	100	100	0	100	100 (5)
<i>N. autotrophica</i>	30	100	100	100	67	0	33	0 (6)
<i>N. hirsuta</i>	11	100	100	100	0	0	0	0 (9)
<i>N. orientalis</i>	21	100	100	100	38	0	43	0 (18)
<i>N. aerocolonigenes</i>	14	100	0	100	50	29	100	0 (14)
<i>N. dassonvillei</i>	31	100	0	0	42	0	10	0 (5)
<i>N. madurae</i>	23	100	0	87	30	87	17	0 (7)
<i>N. pelletieri</i>	21	100	0	57	71	86	0	0 (5)

* Fewer strains were examined for the presence of LCN-A in whole-cell hydrolysates; numbers are indicated in parentheses.

DISCUSSION

We have included all 14 taxa described here in the genus *Nocardia* because many of them are of medical importance and their generic and species identifications should not be beyond the capabilities of the routine diagnostic laboratory (Gordon, 1976). Among the 14 taxa *Saccharopolyspora hirsuta* Lacey & Goodfellow 1975 is the only one not previously assigned to the genus *Nocardia*. Its assignment here is in confirmation of Lacey's (1974) designation of his isolations from bagasse as *Nocardia* spp.

The strains of *N. vaccinii*, *N. transvalensis*, *N. orientalis* and *N. aerocolonigenes* marked with an asterisk in Table 1 are generally accepted as the nomenclatural type strains of the respective taxa. However, the type strain of *N. carnea* has been lost and a neotype strain has not been designated. We propose, therefore, that ATCC 6847 (NCTC 3527), which has long been available, be accepted as the neotype strain of *N. carnea*.

The fore-mentioned physiological and biochemical characteristics of strains belonging to the five aggregates, i.e. *N. carnea*, *N. vaccinii*, *N. transvalensis*, *N. orientalis* and *N. aerocolonigenes*, differentiate them from the well known species, *N. asteroides*, *N. caviae*, *N. brasiliensis*, *N. dassonvillei*, *N. madurae* and *N. pelletieri*, and also from the lesser known *N. amarae*, *N. autotrophica* and *N. hirsuta*. However, only the availability of more strains from different sources and a detailed study of their properties will establish the status of the aggregates as distinct species.

As Tables 2 and 4 attest, within the limits of our methods, nearly every property of a species may vary, especially when a large number of strains are examined. Nor is microbial variation limited to species. Properties used for demarcation of genera may also vary. Tests for the presence of meso-DAP, suggested by Cummins & Harris (1958) as having taxonomic value in describing the genus *Nocardia* and subsequently widely used, were negative for 10 % of our strains of *N. asteroides* and for 5 % of the strains of *N. caviae* (Table 4). Tests for the presence of madurose, used by Meyer (1976) in a generic separation of *N. madurae* and *N. pelletieri* from *N. dassonvillei*, were positive for 87 % of our strains of *N. madurae*, 86 % of the strains of *N. pelletieri* and 29 % of the strains of *N. aerocolonigenes*. The production of aerial hyphae, a characteristic used by Gordon (1976) to define the genus *Nocardia*, varied among the strains described here. Five strains of *N. aerocolonigenes* did not form aerial hyphae, although some, for example the type strain (ISP 5034), did when first isolated. Because the other properties (morphological, physiological and chemotaxonomic) of these strains without aerial hyphae were those of *N. aerocolonigenes*, we did not hesitate to accept them as strains of this species. Their similarities far outweighed their differences.

This investigation was supported in part by grants from the Charles and Johanna Busch Memorial Fund, the Schering-Plough Foundation and the Merck Company Foundation; we are very grateful for this assistance. We also wish to thank the many investigators who have given us their strains.

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