A Piecemeal Description of *Streptomyces griseus* (Krainsky) Waksman and Henrici

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SUMMARY

Forty-six of the 48 strains named *Streptomyces griseus* in our collection have in common a distinctive pattern of properties and are believed to represent a recognizable taxonomic group, or aggregate, of this polytypic species. Seventy-seven additional strains bearing 51 different species names were found to possess the same pattern of characteristics. The distinguishing properties of this aggregate are described and compared with those of *S. albus*, *S. fradiae* and *S. somaliensis*.

INTRODUCTION

Early in this study of the streptomycetes wide variation was observed among cultures of individual strains not only in their pigmentation but also in their formation of aerial hyphae, sporulation and the fragmentation of their substrate hyphae (Gordon & Mihm, 1962). Certain physiological properties, on the other hand, were shared by the parent strains and their soft fragmenting variants. Because the basic taxonomic unit (designated here by the old-fashioned term 'species') can be recognized in different ways, a search was begun for a group of relatively stable characters by which each species might be described and differentiated from the other species of streptomycetes and nocardias in our collection.

According to the criteria investigated, certain species, typified by *Streptomyces fradiae*, were comparatively homogeneous and distinct; either all the strains, or nearly all the strains, gave the same reaction to each test applied (Gordon, 1967). A distinguishing pattern of characters for recognizing *S. fradiae* was thus easily found. Other species were more heterogeneous, or polytypic, and were, apparently, composed of aggregates of strains with intermediate strains connecting the aggregates. *S. griseus* (Krainsky, 1914) Waksman & Henrici (1948) is an example of a polytypic species, and its composition, as represented in our collection, confirmed Pridham's (1964) description of *S. griseus* as a 'species comprising many sub-species'.

On the basis of physiological properties, 46 of the 48 strains named *Streptomyces griseus* in the Institute of Microbiology, Rutgers, The State University, New Jersey (IMRU), collection were relatively similar and are representative of a single aggregate joined to other aggregates by intermediate strains. These 46 strains have been kept as separate strains, although some are duplicates maintained for several years in other laboratories and some are variants developed for specific purposes. The pattern of reactions by which these 46 strains can be recognized, and a list of other strains in this IMRU collection with the same pattern of characters are presented.

METHODS

The 123 strains listed in Tables 1 and 2 and the strains examined by Gordon (1966, 1967) were compared by means of observations and tests described in the same reports. In addition, the following tests were used.

Hydrolysis of hippurate. Cultures were prepared in hippurate broth (tryptone, 10 g.; beef extract, 3 g.; yeast extract, 1 g.; glucose, 1 g.; Na_2HPO_4 , 5 g.; Na hippurate, 10 g.; distilled water, 1000 ml.). After 6 weeks of incubation at 28°, 1 ml. of each culture, as free from clumps of growth as possible, was mixed with 1.5 ml. of 50% (v/v) sulphuric acid. The appearance of finely divided crystals in the acid mixture after 4 hr at room temperature indicated the presence of benzoic acid (Baird-Parker, 1963). When the identity of the crystals was in doubt, their melting point was compared with that of benzoic acid.

Oxidation of glucose. The oxidative or fermentative utilization of glucose was determined by the Hugh & Leifson (1953) test. The basal medium (peptone, 2 g.; NaCl, 5 g.; K_2HPO_4 , 0.3 g.; agar 3 g.; 0.04% (w/v) aqueous solution of bromocresol purple, 15 ml.; distilled water, 1000 ml.; pH 7.1) was tubed (7.5 ml./16 mm. diam. tube), autoclaved and quickly cooled. Before the agar solidified, 0.5 ml. of 15% (w/v) aqueous solution of glucose, also sterilized by autoclaving, was added to each tube. As soon as the agar hardened, duplicate tubes were inoculated by stabbing from a 10- to 14-day culture in glucose broth; one tube was then sealed with a mixture of petroleum jelly and paraffin (paraffin, 60% (w/w); petroleum jelly, 40% (w/w)). During the first 24 hr of incubation, some cultures had to be resealed by heating the glass around the jelly in a small flame. The cultures were incubated at 28° and observed at 7 and 28 days. A culture that grew both in the aerobic and the anaerobic tubes, but formed the acid colour of the indicator only in the aerobic tube was recorded as oxidizing glucose. A culture that grew and formed the acid colour of the indicator both in the aerobic and the anaerobic tubes was recorded as fermenting glucose. A culture that grew in both tubes but did not change the colour of the indicator was recorded as not attacking glucose in the medium of Hugh & Leifson.

Resistance to salicylate. A tube of glucose broth (peptone, 5 g.; beef extract, 5 g.; yeast extract, 5 g.; glucose, 5 g.; distilled water, 1000 ml.) and a tube of glucose broth containing 0.2% (w/v) Na salicylate were inoculated from a 10- to 14-day culture in glucose broth with a loop 2 mm. outside diameter. The tubes were examined immediately and those containing inoculum that might be mistaken for growth were replaced. Growth of the cultures was observed after 7 and 28 days of incubation at 28°. This test was suggested by the method of Tsukamura (1962).

Utilization of benzoate, lactate and oxalate. Modifications of Koser's (1924) citrate agar were made by adding 2 g. Na benzoate, Na lactate or Na oxalate to: 1 g. NaCl, 0.2 g. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g. $(\text{NH}_4)_2\text{HPO}_4$, 0.5 g. KH_2PO_4 , 15 g. agar, 1000 ml. distilled water, 20 ml. 0.4% (w/v) phenol red solution. The pH value of each medium was adjusted to ensure a slightly acid colour of the indicator after autoclaving. Cultures on slopes of each medium were observed for the alkaline colour of the indicator after 7 and 28 days of incubation at 28°.

Determination of diaminopimelic acid (DAP). The method of Becker, Lechevalier, Gordon & Lechevalier (1964) was used to establish the form of DAP in hydrolysates of the whole organisms.

RESULTS

After 5 days of incubation, cultures of the strains (Tables 1, 2) grown on glycerol agar and stained by a modified Ziehl-Neelsen method were composed of short or long slender branching filaments that did not retain the carbol fuchsin. In some cultures the filaments were vacuolated, in others they were broken into uneven fragments or bacillary forms. Spores, when formed on the glycerol agar, appeared as spherical or oval bodies.

Table 1. *Reference strains of Streptomyces griseus* (Krainsky)
Waksman & Henrici

Laboratory no.	Source, strain name or number
3326	N.J. Agr. Exp. Station (Waksman & Curtis 360)
3326a	Centraalbureau voor Schimmelcultures (CBS) (Waksman & Curtis 360)
3326b	A. Kelner, Biological Lab., Cold Spring Harbor, N.Y. (mutant 1 of Waksman & Curtis 360)
3378	F. A. Wolff, Duke Univ. (Yucatan)
3463, 3464	N.J. Agr. Exp. Station (18-16, soil; D-1, chicken's throat)
3464a	N.J. Agr. Exp. Station (sporulating variant of D-1)
3475	H. W. Anderson, Univ. of Ill. (variant 42.1 of 4)
3478, 3481	N.J. Agr. Exp. Station (25-G, peat; 22, soil)
3480	Schenley Labs., Inc., Lawrenceberg, Ind. (SL-842)
3492-3494, 3498	N.J. Agr. Exp. Station (variants 1, 9, 19 and C ₁ of 18-16)
3496, 3495	N.J. Agr. Exp. Station (4 and rhodomycetin producing variant of 4)
3499	C. H. Reilly, N.J. Agr. Exp. Station (C3, a streptomycin producing variant of D-1)
3510	W. Garson, N.J. Agr. Exp. Station (HF, cow manure)
3511	Parke, Davis and Co., Detroit (04506)
3512	N.J. Agr. Exp. Station (2 PR, a phage-resistant variant of 3475)
3523, 3524	Merck and Co., Rahway, N.J. (7R2557 and unnumbered); CBS 3496 and 3493)
3527	N.J. Agr. Exp. Station (Mexican soil)
3533	A. B. Kupferberg, N.J. Agr. Exp. Station (31)
3536-3539	H. Umezawa, Nat. Inst. of Health, Tokyo (S-1, S-2, S-3 and S-4)
3546	F. Bustinza, Univ. of Madrid (Z)
3563	W. Gold, N.J. Agr. Exp. Station; Univ. of Wis.
3567a, 3567b	N.J. Agr. Exp. Station (intestine of earthworm)
3570	H. A. Lechevalier, N.J. Agr. Exp. Station (C 135, produces candididin)
3586-3589	R. S. Weiser, Univ. of Wash. (214, 305, 1260 and 2017)
3616	P. P. DeSomer, Rega Inst., Louvain (R 33, a variant of 3464)
3620, 3621	H. E. Bailey, Wayne Univ. (103 and 105)
3646	R. G. Benedict, U.S. Dept. Agr., Peoria, Ill. (NRRL B-683)
3734	J. Ehrlich, Parke, Davis and Co., Detroit (A 4903-PD 04797-NRRL 2426)
3754	H. B. Woodruff, Merck and Co., Rahway, N.J. (L-118 MA-45)
3903	H. A. Lechevalier, Rutgers Univ. (LP-16, produces geosmin)
A10137	American Type Culture Collection (ATCC) (10137); S.A. Waksman (4)

On soil extract and Bennett agars, the colonies were densely or loosely filamentous. The substrate hyphae were extensive, fine, wavy, branching and intertwined with the substrate hyphae of adjacent colonies. The aerial hyphae varied from sparse to abundant, straight to loosely coiled, and short to long, intertwining sometimes with the aerial hyphae of adjacent colonies. The aerial hyphae of some cultures branched in clusters or whorls. During the 14-day incubation chains of bead-like spores were formed.

Macroscopically the cultures on yeast dextrose agar at 7 days were abundant and spreading and finely or coarsely wrinkled or flat. The growth was cream-

Table 2. *Strains identified as Streptomyces griseus (Krainsky, 1914) Waksman & Henrici (1948)*

Laboratory no.	Name when received, source, and strain name or number
3845	<i>Actinomyces cyaneofuscatus</i> Kudrina (1957); G. F. Gauze, Inst. of Antibiotics, Moscow (92/54)
413	<i>A. keratolytica</i> ; J. M. Coffey, N.Y. State Dept. of Health (5229)
3765	<i>A. longissimus</i> Krassil'nikov (1941); H. J. Kutzner, Inst. f. Biochemie des Bodens, Braunschweig-Volkenrode, Germany (116); M. S. Todorovič
3347	* <i>A. roseus</i> Krainsky (1914); N.J. Agr. Exp. Station
3007	<i>A. sulphureus</i> ; N.J. Agr. Exp. Station
506	<i>Nocardia alba</i> ; T. Thibault, Inst. Pasteur, Paris
N 3258, N 4520	<i>N. asteroides</i> ; NCTC (3258, 4520)
3301	<i>N. farcinica</i> ; N.J. Agr. Exp. Station; CBS
676	<i>N. haemolytica</i> ; E. N. Azarowicz, Univ. of Calif. (19)
3856	<i>N. italica</i> Spalla, Amici & Bianchi (1961); A. DiMarco, Farmitalia, Milan (soil)
A 6855	<i>N. leishmani</i> ; ATCC (6855); BCTC (658)
A 6245	<i>N. madurae</i> ; ATCC (6245); C. W. Emmons (9902)
505	<i>N. odorifera</i> (Rullmann, 1895) Castellani & Chalmers (1913); P. Thibault
A 4919	<i>N. sylvodorifera</i> ; ATCC (4919); A. Castellani
511	<i>N. violacea</i> (Rossi-Doria, 1891) Chalmers & Christopherson (1916); P. Thibault
638, 712, 713	<i>Nocardia</i> spp.; D. B. Tilden, Chicago Zoological Park (lung of a kangaroo, lung lesion of a sitatunga, lung of a black-tailed deer)
699	<i>Nocardia</i> sp.; C. W. Emmons, Nat. Inst. of Health, Bethesda, Md. (9940); A. L. Carrion (mycetoma)
3049	<i>Streptomyces acidoresistans</i> ; N.J. Agr. Exp. Station; Král Collection
3861	<i>S. albidoflavus</i> (Rossi-Doria, 1891) Waksman & Henrici (1948); P. M. Arnow, Rutgers Univ.; H. Okada
N 7807	<i>S. albus</i> ; NCTC (7807); W. R. Maxted
3005, 3006	<i>S. albus</i> ; N.J. Agr. Exp. Station; CBS; H. W. Wollenweber (potatoes)
3019	<i>S. albus</i> ; N.J. Agr. Exp. Station; CBS
3361	<i>S. albus</i> ; N.J. Agr. Exp. Station
3448	<i>S. albus</i> ; M. Welsch, Univ. de Liège
3462	<i>S. albus</i> ; C. H. Meredith, Glenleigh Lab., Highgate, Jamaica, B.W.I. (11, anatonistic to <i>Fusarium oxysporum cubense</i>)
3307	<i>S. annulatus</i> ; N.J. Agr. Exp. Station; CBS
3309A	<i>S. aureus</i> (Waksman & Curtis, 1916) Waksman & Henrici (1948); N.J. Agr. Exp. Station
3811	<i>S. baarnensis</i> Pridham, Hesseltine & Benedict (1958); Lederle Labs., Pearl River, N.Y.; CBS
3312	<i>S. californicus</i> (Waksman & Curtis, 1916) Waksman & Henrici (1948); N.J. Agr. Exp. Station
3593	<i>S. californicus</i> ; H.B. Woodruff, Merck and Co., Inc. Rahway, N.J. (51 R 3639)
A 4879	<i>S. candidus</i> ; ATCC (4879); S. A. Scudder (23314)
3416	<i>S. candidus</i> ; N.J. Agr. Exp. Station; ATCC (4878)
3758	<i>S. cavourensis</i> Waksman (1961); R. Craveri, S. A. Montecatini, Milan (829, Linate)
3313A, 3313B	<i>S. cellulosa</i> (Krainsky, 1914) Waksman & Henrici (1948); N.J. Agr. Exp. Station (variants of 3313)
3657	<i>S. chrysomallus</i> Lindenbein (1952); K. B. Raper, U.S. Dept. Agr., Peoria, Ill. (NRRL 2250); Schenley Labs., Inc.
3036	<i>S. erythaeus</i> (Waksman, 1923) Waksman & Henrici (1948); CBS
3316	<i>S. exfoliatus</i> (Waksman & Curtis, 1916) Waksman & Henrici (1948); CBS (Waksman & Curtis)
3671	<i>S. flavochromogenes</i> (Krainsky, 1914) Waksman & Henrici (1948); Heyden Chemical Corp., Princeton, N.J. (8)
3590	<i>S. gougeroti</i> (Duché, 1934) Waksman & Henrici (1948); CBS
3068	<i>S. griseobrunneus</i> Waksman (1961); N. J. Agr. Exp. Station (218)
3325	<i>S. griseolus</i> (Waksman, 1923) Waksman & Henrici (1948); N.J. Agr. Exp. Station
3328	<i>S. halstedii</i> (Waksman & Curtis, 1916) Waksman & Henrici (1948); N.J. Agr. Exp. Station
3329	<i>S. intermedius</i> ; H. W. Wollenweber, Forschungs Inst. f. Kartoffelbau, Berlin

Table 2. (contd.)

Laboratory no.	Name when received, source and strain name or number
3330	<i>S. lavendulae</i> ; N.J. Agr. Exp. Station
3331	<i>S. lipmannii</i> (Waksman & Curtis, 1916) Waksman & Henrici (1948); N.J. Agr. Exp. Station
3332	<i>S. microflavus</i> (Krainsky, 1914) Waksman & Henrici (1948); CBS; A. Krainsky
3334	<i>S. odorifer</i> (Rullmann, 1895) Waksman (1953); N.J. Agr. Exp. Station; Král Collection
3677	<i>S. parvulus</i> Waksman & Gregory (1954); F. J. Gregory, N.J. Agr. Exp. Station (G-375)
3686	<i>S. praeus</i> (Krainsky, 1914) Waksman & Henrici (1948); R. G. Benedict, U.S. Dept. Agr., Peoria, Ill. (NRRL B-1455)
3374	<i>S. praecox</i> (Millard & Burr, 1926) Waksman (1953); W. A. Millard, Univ. of Leeds
3344	<i>S. reticuli</i> (Waksman & Curtis, 1916) Waksman & Henrici (1948); N.J. Agr. Exp. Station
3689	* <i>S. roseochromogenus</i> (Jensen, 1931) Waksman & Henrici (1948); R. W. Thoma, E. R. Squibb and Sons, New Brunswick, N.J. (SC 1624)
3350	<i>S. rutgersensis</i> (Waksman & Curtis, 1916) Waksman & Henrici (1948); N.J. Agr. Exp. Station
3371	<i>S. sampsonii</i> (Millard & Burr, 1926) Waksman (1953); W. A. Millard
3351	<i>S. saprophyticus</i> ; N.J. Agr. Exp. Station
3029	<i>S. scabies</i> (Thaxter, 1892) Waksman & Henrici (1948); N.J. Agr. Exp. Station; M. Shapovalov (Morse 17)
3352	<i>S. scabies</i> ; CBS
A 10246	<i>S. scabies</i> ; ATCC (10246); W. H. Burkholder (N 54); R. W. Goss
3375	<i>S. setonii</i> (Millard & Burr, 1926) Waksman (1953); W. A. Millard
N 3236	<i>S. somaliensis</i> ; NCTC (3236); Wellcome Tropical Research Labs. (mycetoma)
3418	<i>S. sylvodoriferus</i> ; N.J. Agr. Exp. Station; ATCC (4919)
3367	<i>S. tetanusemus</i> ; N.J. Agr. Exp. Station; Pribram Collection
A 11861	* <i>S. vinaceus</i> Waksman (1953); ATCC (11861); NRRL (B-2285)
3372	<i>S. viridis</i> (Millard & Burr, 1926) Waksman (1953); W. A. Millard
3690	<i>S. viridis</i> ; R. W. Thoma (SC 1633)
A 6867	<i>S. willmorei</i> (Erikson, 1935) Waksman & Henrici (1948); ATCC (6867); NCTC (1856); J. G. Willmore
581	<i>Streptomyces</i> sp.; C. C. Campbell, Army Med. Service Graduate School, Washington, D.C. (698)
985	<i>Streptomyces</i> sp.; T. G. Pridham, U.S. Dept. of Agr., Peoria, Ill. (D)
3069	<i>Streptomyces</i> sp.; N.J. Agr. Exp. Station (142)
3482	<i>Streptomyces</i> sp.; R. L. Mayer, Ciba Pharmaceutical Co., Summit, N.J.
N 4523	<i>Streptothrix buccalis</i> Goadby (1903); NCTC (4523); ATCC (3311); Pribram Collection
3311	<i>Streptothrix buccalis</i> ; N.J. Agr. Exp. Station; C.-E. A. Winslow

* Illegitimate specific epithet.

coloured, yellow, greenish yellow, olive drab or brown. Some cultures did not form aerial hyphae visible to the unaided eye; others showed patches or a thin coating of whitish aerial hyphae; still others were abundantly covered with greyish white or grey aerial hyphae. Soluble pigments were greenish yellow, greenish brown, reddish brown or red (Gordon & Horan, 1968, pl. 2).

Properties (Table 3) shared by all 46 reference strains of *Streptomyces griseus*, or those shared by a high percentage of the strains were: decomposition of adenine, casein, hypoxanthine, tyrosine, urea and xanthine; lack of growth at 45°; growth at 10°; survival after 8 hr at 50°; use of citrate, lactate, malate and succinate as sole carbon source; failure to utilize benzoate, mucate or oxalate; sensitivity to lysozyme and

Table 3. *Pattern of reactions of Streptomyces griseus*

Property	46 reference strains	77 similar strains
	(% positive strains)	
Decomposition of		
Adenine	96	100
Casein	100	100
Hypoxanthine	100	100
Tyrosine	100	100
Urea	96	97
Xanthine	100	100
Growth at		
45°	0	0
40°	13	45
10°	100	100
Survival at 50° for 8 hr	100	99
Nitrite from nitrate	83	68
Utilization of		
Benzoate	0	0
Citrate	100	99
Lactate	93	88
Malate	100	100
Mucate	0	0
Oxalate	0	0
Succinate	100	100
Resistance to		
Lysozyme	2	0
Salicylate	0	3
Oxidation of glucose	100	97
Fermentation of glucose	0	0
Hydrolysis of		
Hippurate	2	6
Starch	100	100
Acid from		
Adonitol	57	31
Arabinose	41	75
Dulcitol	0	0
Erythritol	0	1
Galactose	100	99
Glucose	100	100
Inositol	20	13
Lactose	100	100
Maltose	100	100
Mannitol	98	96
Mannose	100	100
Melibiose	0	0
α -Methyl-D-glucoside	100	97
Raffinose	2	3
Rhamnose	24	34
Sorbitol	0	0
Trehalose	100	99
Xylose	100	100

salicylate; oxidation of glucose; inability to form benzoic acid from hippurate; hydrolysis of starch; formation of acid from galactose, glucose, lactose, maltose, mannitol, mannose, α -methyl-D-glucoside, trehalose and xylose; failure to attack dulcitol, erythritol, melibiose, raffinose or sorbitol. Variable characteristics of the 46

Table 4. *Comparison of properties of four species of streptomyces*
(% positive strains)

Property	<i>S. griseus</i> (123 strains)	<i>S. albus</i> (23 strains)	<i>S. fradiae</i> (23 strains)	<i>S. somaliensis</i> (22 strains)
Decomposition of				
Adenine	98	4	100	0
Casein	100	100	100	100
Hypoxanthine	100	100	0	0
Tyrosine	100	100	100	100
Urea	97	100	0	0
Xanthine	100	83	0	0
Growth at				
50°	0	100	0	0
45°	0	100	91	55
40°	33	100	100	91
10°	100	0	5	9
Survival at 50° for 8 hr	99	100	91	50
Nitrite from nitrate	73	60	100	0
Utilization of				
Citrate	99	100	0	18
Lactate	90	100	26	0
Malate	100	100	95	37
Mucate	0	0	0	0
Oxalate	0	57	0	0
Succinate	100	100	100	45
Resistance to				
Lysozyme	1	21	0	0
Salicylate	2	100	0	0
Oxidation of glucose	98	96	100	0
Fermentation of glucose	0	4*	0	0
Hydrolysis of				
Hippurate	5	30	0	5
Starch	100	0	100	36
Acid from				
Adonitol	43	100	0	0
Arabinose	63	17	91	0
Dulcitol	0	0	0	0
Erythritol	1	100	0	0
Galactose	99	100	100	0
Glucose	100	100	100	50
Inositol	15	65	0	0
Lactose	100	100	63	0
Maltose	100	100	100	64
Mannitol	97	100	0	0
Mannose	100	100	100	5
Melibiose	0	13	0	0
α-Methyl-D-glucoside	98	100	0	0
Raffinose	2	0	0	0
Rhamnose	31	0	0	0
Sorbitol	0	4	0	0
Trehalose	99	91	100	0
Xylose	100	100	100	0

reference strains were: growth at 40°; reduction of nitrate or nitrite; acid production from adonitol, arabinose, inositol, and rhamnose.

The following 21 strains (Tables 1, 2) were found to contain *LL*-diaminopimelic acid: 3029, 3068, 3475, 3523, 3527, 3567a, 3567b, 3856, N3236, N3258, N4520, N4523,

N7808, 413, 505, 506, 511, 638, 676, 712 and 713. The 101 remaining strains of *Streptomyces griseus* were not tested for the form of diaminopimelic acid.

The reactions of the 77 strains listed in Table 2 are given in Table 3, and in Table 4 these reactions are combined with those of the 46 reference strains to represent one aggregate of *Streptomyces griseus*. The pattern of characters of these strains is compared (Table 4) with those of the strains in our collection of *S. albus* (Rossi-Doria) Waksman & Henrici, *S. fradiae* (Waksman & Curtis) Waksman & Henrici and *S. somaliensis* (Brumpt) Waksman & Henrici. As a group the following criteria served to differentiate this aggregate of *S. griseus* from the three other species of streptomycetes: decomposition of adenine, hypoxanthine, urea, and xanthine; growth at 10° but not at 45°; use of citrate; sensitivity to salicylate; oxidation of glucose; hydrolysis of starch; acid production from galactose, lactose, mannitol, mannose, α -methyl-D-glucoside, trehalose and xylose; and lack of acid formation from erythritol.

DISCUSSION

The usual practice of author citation of the name under which each strain was received was difficult to follow. After reading the available original descriptions of the species named in Table 2, we attempted to decide whether or not each strain was representative of its original strain. Because many of the descriptions of these strains relied on variable properties, and because of human fallibility in the preparation of descriptions and in the maintenance of cultures, the task proved to be impossible. We accepted, therefore, all strains whose authenticity was not definitely challenged either by the first description or by the recognition of very different strains as representatives of the particular species. For example, a strain deposited in this collection as *Nocardia madurae* and subsequently found to be *Streptomyces griseus* was obviously not typical of *N. madurae*, because other very different strains are accepted as representing *N. madurae*.

The names of the strains in Table 2 that are followed immediately and without intervening semicolon by the name of the author(s) are regarded as synonymous with *Streptomyces griseus*. Some of these strains came from the author(s) of the species. Other strains whose histories cannot be traced to the describer of the species may, however, be typical of the original strains, since they were found to possess characters that do not appear to be at variance with the original species descriptions.

Strain 3347 was accepted as conforming to Krainsky's (1914) description of *Actinomyces roseus*, although previously Namyslowski (1912) had given the name to another strain. In 1916, Chalmers & Christopherson assigned Krainsky's *A. roseus* to the genus *Nocardia*. *N. roseus* has priority therefore over *A. roseochromogenus*, a name assigned to Krainsky's *A. roseus* by Jensen (1931). The legitimate name for Krainsky's species is *Streptomyces roseus* (Chalmers & Christopherson) Pridham, Hesseltine & Benedict (1958). Mayer *et al.* (1951) named ATCC 11861 *S. vinaceus* but did not describe it; the necessary description came from Waksman (1953). Although ATCC 11861 is considered as authentic, the name *S. vinaceus* is considered an illegitimate one for the species because Jones (1951) gave the name *S. vinaceus* to another strain.

Beijerinck (1912), cited by Waksman (1953), used the name *Actinomyces (Streptothrix) annulatus*, but since he did not describe the strain, his name has no nomenclatural

status. Although Wollenweber (1920) ascribed the name to Krainsky, he gave no reference to the Krainsky work, and we have been unable to find it. Wollenweber (1920) also described and named some isolates of his own *A. annulatus*; in our opinion he was correctly cited by Hütter (1963) as the original author of the species name.

We are unable to give the authority for the name borne by ATCC 4879, because this strain and strain 4878 were deposited in the ATCC in 1933 by S. A. Scudder and appear in the ATCC catalogue as *Actinomyces candidus* Petruschky (1903). Waksman (1953) assigned *A. candidus* Krassil'nikov (1941), which he did not regard the same as *Streptothrix candida* Petruschky, to the genus *Streptomyces* and in 1961 designated ATCC 4878 (IMRU 3416) as the type strain of *S. candidus* (Krassil'nikov) Waksman.

Waksman (1953) cited Krüger (1809) as the author of *Oospora intermedia*, but Krüger did not mention this name in his report. Wollenweber (1920) named and described some isolations of his own as '*Actinomyces intermedius* (Krüg.) Wr.' but did not identify Krüger's paper. Kudrina's (1957) and Pridham's (1964) listing of Wollenweber as the authority for the specific epithet is therefore in accord with the rules of nomenclature.

Actinomyces saprophyticus was named by Gasperini (1892); later Chalmers & Christopherson (1916) assigned the same specific epithet to a species in the genus *Nocardia*. An authority for the transfer of either species to the genus *Streptomyces* was not found. A search of the literature did not reveal the author of the name and description of *N. hemolytica*, *S. acidoresistans* or *S. tetanusemus*. Strains labelled *N. sylvodorifera* and *S. sylvodorifera* could be traced to Castellani, but Castellani was unable to furnish the reference (personal communication), and the original characterization and naming of this species was not discovered.

Among the strains listed in Table 2, those labelled *Actinomyces sulphureus*, *Nocardia asteroides*, *N. farcinica*, *N. madurae*, *Streptomyces albus*, *S. lavendulae* and *S. somaliensis* were unlike recognized strains of these species and were therefore regarded as mis-named. Strain 413 was not accepted as representing *A. keratolytica* because Acton & McGuire (1931) characterized their species as forming limpet-shaped colonies, black or red in colour, with deep roots penetrating the media, and as resembling, in certain respects, cultures of *A. bovis* and *A. madurae*. ATCC 6855 was unacceptable as *N. leishmani* Chalmers & Christopherson (1916), a species originally described as acid-fast by Birt & Leishman (1902).

A number of the strains listed in Table 2 were previously recognized as strains of *Streptomyces griseus* by Baldacci & Comaschi (1956), Benedict *et al.* (1955), Burkholder, Sun, Anderson & Ehrlich (1955), Ettlinger, Corbaz & Hütter (1958), Hütter (1963), Krassil'nikov (1949), Pridham (1964), and others. Our findings, based mainly on different observations, support their conclusions.

The pattern of properties presented is for the identification of only one aggregate of strains of the heterogeneous species *Streptomyces griseus* and is not intended as a delination of the species. Strains labelled *S. coelicolor*, for example, which were recognized as *S. griseus* by Hütter (1963), Pridham (1964) and others, have, with three exceptions, the same pattern of characters. The three characters of strains of *S. coelicolor* not shared by the strains of this aggregate of *S. griseus* do not, however, offer a definite separation of the two groups, because other strains possess one or two of the three properties and are clearly intermediate. Other aggregates of strains of *S. griseus* are also represented in our collection and are bound together by inter-

mediate strains. With the exception of the taxonomic group described here, the representation in our collection of the aggregates of *S. griseus* is inadequate, and we are unable to offer at this time a pattern of properties for the identification of the entire species.

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