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Numerical Taxonomy of *Actinomadura* and Related Actinomycetes

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One hundred and fifty-six *Actinomadura* strains, marker strains of related taxa, and related isolates from bagasse and fodder were the subject of numerical phenetic analyses using 90 unit characters. The data were examined using the simple matching (S_{SM}), Jaccard (S_J) and pattern (D_P) coefficients and clustering was achieved using both single and average linkage algorithms. Cluster composition was not markedly affected either by the coefficient or clustering algorithms used or by test error, estimated at 4·5%. *Actinomadura dassonvillei*, *Actinomadura madurae* and *Streptomyces somaliensis* formed good taxospecies, but the separation of *Actinomadura pelletieri* strains into two clusters by S_J and S_{SM} analysis requires further study. The single representatives of *Actinomadura helvata*, *Actinomadura pusilla*, *Actinomadura roseoviolacea*, *Actinomadura spadix* and *Actinomadura verrucosospora* seemed to form new centres of variation while *Actinomadura citrea* and *Actinomadura malachitica* showed much similarity with *Actinomadura madurae*. Most of the isolates from bagasse and fodder were recovered in two well-defined phena, provisionally labelled clusters 'A' and 'B' which showed little similarity to either *Actinomadura* or *Nocardia* strains. The effect of the different coefficients on the aggregation of clusters is discussed.

INTRODUCTION

The genus *Actinomadura* Lechevalier & Lechevalier 1970 was proposed to accommodate so-called *Nocardia* species with walls containing *meso*-diaminopimelic acid but lacking arabinose and galactose (Becker *et al.*, 1965). Initially, three species, *Nocardia dassonvillei* (Gordon & Horan, 1968), *Nocardia madurde* and *Nocardia pelletieri* (Gordon, 1966), were transferred to *Actinomadura*, which was classified in the family Thermoactinomycetaceae. Later, the genus was transferred to the family Thermomonosporaceae because endospores were not produced (Cross & Goodfellow, 1973). The separation of *Actinomadura* from *Nocardia* has subsequently been supported by numerical phenetic (Tsukamura, 1969; Goodfellow, 1971), DNA–DNA reassociation (Mordarski *et al.*, 1977), phage typing (Prauser, 1976), chemical (Goodfellow & Minnikin, 1978) and serological (Goodfellow *et al.*, 1974) data. However, the 8th edition of *Bergey's Manual of Determinative Bacteriology* (McClung, 1974) describes *Actinomadura* as a genus *incertae sedis*, thus perpetuating the unsettled taxonomic history of the species it contains (Lacey *et al.*, 1978).

Further *Actinomadura* species have been recognized on the basis of morphology and wall chemotype (Lacey *et al.*, 1978); consequently little is known of the relationships of these taxa to one another or to the three originally described species (Lechevalier & Lechevalier, 1970). In a numerical taxonomic survey, however, *Actinomadura dassonvillei* strains were only loosely associated with clusters corresponding to *Actinomadura madurae* and *Actinomadura pelletieri* although all three species formed an aggregate taxon equivalent in rank to

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clusters equated with *Nocardia*, *Oerskovia* and *Rhodococcus* (Goodfellow, 1971). *Actinomadura dassonvillei* can be distinguished from the other two species on the basis of spore morphology (Williams *et al.*, 1976), fatty acid composition (Agre *et al.*, 1975), pigmentation (Lechevalier *et al.*, 1971), absence of madurose (3-*O*-methyl-D-galactose; Lechevalier & Gerber, 1970), menaquinone composition (Collins *et al.*, 1977) and polar lipid composition (Minnikin *et al.*, 1977). Meyer (1976) proposed the new genus *Nocardiopsis* for this species.

Classically, actinomadurae have been isolated from clinical material. *Actinomadura madurae* and *A. pelletieri* are primary agents of actinomycete mycetoma in man. Although *A. dassonvillei* was first isolated from mouldy oat grain (Brocq-Rousseu, 1904), it can infect animals and man (Gordon & Horan, 1968; Schaal, 1977). It also occurs in hay, cotton and soil (Lacey *et al.*, 1978). Most newly described species have been isolated from soil (Nonomura & Ohara, 1971; Lavrova *et al.*, 1972) while others, classified provisionally as *Actinomadura* strains on the basis of their morphology (Lacey, 1971, 1978), were isolated from mouldy hay and barley grain. In a subsequent review (Lacey *et al.*, 1978), however, the affinities of these isolates were considered to be uncertain.

In the present study, representatives of *Actinomadura* spp., marker cultures of *Nocardia* spp. and *Streptomyces somaliensis*, and '*Actinomadura*' isolates from mouldy hay and fodder were compared using numerical phenetic methods in an attempt to clarify the intra- and supra-generic relationships of *Actinomadura*.

METHODS

Strains. The 156 test strains (Table 1) were maintained on glucose yeast extract agar (GYEA) at room temperature (Gordon & Mihm, 1962).

Isolation of 'Actinomadura' strains. These strains were isolated from air, bagasse and fodder using a wind-tunnel technique (Lacey, 1971, 1974); the Anderson sampler was loaded with Petri dishes containing half-strength nutrient agar medium supplemented with 50 μg actidione ml^{-1} (Gregory & Lacey, 1963).

Collection of data. Each strain was examined for 90 unit characters (Tables 2 and 3). Media were inoculated from GYEA cultures and incubated at 37 °C for up to 21 d, unless otherwise stated. A few of the '*Actinomadura*' isolates (A53, A62, A66, A79, A81 and A89) that grew poorly, if at all, at 37 °C were incubated at 30 °C. Growth tests were performed in divided polystyrene Replidishes (Sneath & Stevens, 1967); antibiotic sensitivity, colony morphology and degradation tests were done in Petri dishes; and urease, allantoinase and lysozyme activity were examined in test-tubes. Most of the media and methods used have been described elsewhere (Goodfellow, 1971; Lacey & Goodfellow, 1975); details of the remainder are given below.

Colonial characters. Colony morphology and pigmentation were observed on GYEA plates after 7 and 14 d.

Biochemical and degradative tests. Cultures were examined using methods previously described (Goodfellow, 1971; Lacey & Goodfellow, 1975), with most tests being read after 14 d. However, gelatin, starch, DNA and RNA tests were read after 7 d, guanine and cellulose degradation after 21 d and allantoin and urea tests weekly up to 4 weeks. Ribonucleases were detected in a basal medium (20 g tryptone, 5 g NaCl and 15 g agar in 1 l distilled water; at pH 7·3) supplemented with RNA (0·3 %, w/v) (Jeffries *et al.*, 1957).

Testosterone degradation was tested by streaking a dense inoculum of each culture across a plate containing 0·1 % (w/v) testosterone (BDH) in Gordon's (1967) basal medium, and looking for the disappearance of the insoluble testosterone underneath and around the growth.

Organic compounds as sole sources of carbon. Strains were examined for their ability to grow on 42 carbon sources (Tables 2 and 3; Goodfellow, 1971).

Antibiotic sensitivity studies. Eleven antimicrobial agents contained in filter paper discs (Tables 2 and 3) were tested for activity against the strains by the method of Goodfellow & Orchard (1974). Resistance to lysozyme was determined after 28 d (Gordon, 1966).

Coding of data. Nearly all of the characters existed in one of two mutually exclusive states and were scored plus (+) or minus (−). Qualitative multistate characters, such as pigmentation and colony elevation, were scored plus (+) for the character state shown and minus (−) for the alternatives; quantitative multistate characters, for example, aerial hyphae production, were scored by the additive method (Sneath & Sokal, 1974). Sensitivity to antimicrobial agents was scored plus (+). The $n \times t$ table contained data for 156 bacteria and 90 unit characters. The binary data were recorded on standard IBM punch cards.

Computer analysis. Data were examined using the Clustan 1A program (Wishart, 1968) on an IBM370/180

Table 1. *Designation and source of strains assigned to clusters based on pattern differences*

A. *Major clusters*

(*a*) Strains assigned to cluster 1 (*Actinomadura madurae*)

*A138	*Actinomadura citrea*, G. F. Gause, Institute of New Antibiotics, Bolshaia Pirogovskaia 11, Moscow U.S.S.R., INA 1849
A22	*Actinomadura madurae*, M. Mariat, Institut Pasteur, Paris, France, 725
A25	*A. madurae*, M. Mariat, 393
A30	*A. madurae*, M. Mariat, 703; mycetoma, Mexico
A31	*A. madurae*, M. Mariat, 363; madura foot, Tunis
A32	*A. madurae*, M. Mariat, 364; madura foot
†[a]*A113	*A. madurae*, ATCC 19425
A124	*A. madurae*, H. Prauser, Zentralinstitut für Mikrobiologie und Experimentelle Therapie, Jena, DDR, IMET 7144; H. A. Lechevalier; R. E. Gordon, 1136; soil
[b]A125	*A. madurae*, H. Prauser, IMET 7145; H. A. Lechevalier, Sal 1
A126	*A. madurae*, H. Prauser, IMET 7146; H. A. Lechevalier, 1091
A133	*A. madurae*, M. A. Gordon, Department of Health, Albany, New York, U.S.A., 291A
*A139	*Actinomadura malachitica*, G. F. Gause, INA 1920
*A4	*Actinomadura verrucosospora*, H. Nonomura, Faculty of Engineering, Yamanashi University, Kofu, Japan, A184
A74	'*Actinomadura*' sp., J. Lacey, A270; hay
A107	'*Actinomadura*' sp., J. Lacey, A888; air sample, barley silo
[a]*A16	*Nocardia madurae*, NCTC 5654; J. T. Duncan in 1938; madura foot
A17	*N. madurae*, NCTC 1070; J. T. Duncan in 1934; madura foot
[a]*A142	*N. madurae*, NCTC 5654
A11	*Streptomyces madurae*, C. Philpot, London School of Hygiene and Tropical Medicine, 393
A12	*S. madurae*, C. Philpot, 373
A38	*S. madurae*, CBS 331.54
A41	*S. madurae*, CBS 134.65
A43	*S. madurae*, CBS 254.58

(*b*) Strains assigned to cluster 2 (*Actinomadura dassonvillei*)

A114	*Actinomadura dassonvillei*, laboratory strain
[c]*A118	*A. dassonvillei*, H. Prauser; R. E. Gordon, IMRU 509
A119	*A. dassonvillei*, H. Prauser; R. E. Gordon, IMRU 714
[d]A120	*A. dassonvillei*, H. Prauser; R. E. Gordon, IMRU 1250
A121	*A. dassonvillei*, H. Prauser, IMET 9563; J. Meyer, 828.52; soil, Beirut, Lebanon
A129	*A. dassonvillei*, M. A. Gordon, 289G
A49	'*Actinomadura*' sp., J. Lacey, A286; barley grain
[c]*A14	*Nocardia dassonvillei*, NCTC 10488; R. E. Gordon, IMRU 509
[d]A15	*N. dassonvillei*, NCTC 10489; R. E. Gordon, IMRU 1250; erosive plantar disease
A92	*N. dassonvillei*, W. A. Causey, Center for Disease Control, Atlanta, Ga., U.S.A., CDC W2536; drainage from hip
A93	*N. dassonvillei*, W. A. Causey, CDC N249; mycetoma, Colorado
A95	*N. dassonvillei*, W. A. Causey, CDC N2491; source unknown
A112	*N. dassonvillei*, W. A. Causey, CDC N291; cow
[c]A141	*N. dassonvillei*, NCTC 10488

(*c*) Strains assigned to cluster 3 (*Actinomadura pelletieri*)

A132	*Actinomadura madurae*, M. A. Gordon; H. M. Cameron, Nairobi, Kenya
A23	*Actinomadura pelletieri*, M. Mariat, 374; Dakar, 1957
[e]A24	*A. pelletieri*, M. Mariat, 385
A33	*A. pelletieri*, M. Mariat, 326
A35	*A. pelletieri*, M. Mariat, 308
A36	*A. pelletieri*, M. Mariat, 381
A115	*A. pelletieri*, laboratory strain
A128	*A. pelletieri*, laboratory strain
A130	*A. pelletieri*, M. A. Gordon, 295; N. F. Conant
A131	*A. pelletieri*, M. A. Gordon; H. M. Cameron, Nairobi, Kenya
[f]*A19	*Nocardia pelletieri*, NCTC 4162; J. T. Duncan in 1933; E. C. Smith in 1928; mycetoma of arm
[g]A20	*N. pelletieri*, NCTC 9999; R. E. Gordon, 513 in 1957; P. Thibault in 1923
A21	*N. pelletieri*, NCTC 10000; R. E. Gordon 687 in 1957; L. Ajello
A96	*N. pelletieri*, W. A. Causey, CDC N242; Dr Anwar, Pakistan; mycetoma
A97	*N. pelletieri*, W. A. Causey, CDC N86; N. F. Conant, 989
[h]A123	*N. pelletieri*, H. Prauser, IMET 7141; V. D. Kuznetsov, RIA 476
[f]*A143	*N. pelletieri*, NCTC 4162
[g]N49	*N. pelletieri*, R. E. Gordon, Rutgers University, New Brunswick, U.S.A., 513
[h]A100	*Nocardia* (*Proactinomyces*) *pelletieri*, V. D. Kuznetsov, Research Institute for Antibiotics, Moscow, U.S.S.R., RIA 476
A7	*Streptomyces pelletieri*, C. Philpot
A8	*S. pelletieri*, C. Philpot, 3885

Table 1 (*cont.*)

A9 *S. pelletieri*, C. Philpot, 368
A10 *S. pelletieri*, C. Philpot, 1065; E. C. Smith in 1929; arm of patient
A13 *S. pelletieri*, C. Philpot, 388H
A37 *S. pelletieri*, CBS 708.70
A39 *S. pelletieri*, CBS 436.57
A42 *S. pelletieri*, CBS 294.64
A116 *S. pelletieri*, D. Frey, Institute of Medical Research, Crow's Nest, N.S.W., Australia, RNSH 203
[e]A117 *S. pelletieri*, D. Frey, RNSH 2022; Dr Segretain, IP 385
A122 *S. pelletieri*, H. Prauser, IMET 9592
N281 *S. pelletieri*, I. G. Murray, London School of Hygiene and Tropical Medicine, 1065

(*d*) Strains assigned to cluster 4 (*Streptomyces somaliensis*)

A18 *Nocardia pelletieri*, NCTC 3026; J. T. Duncan in 1929; mycetoma
A26 *Streptomyces somaliensis*, M. Mariat, 395; Fort Lamy, Chad
A27 *S. somaliensis*, M. Mariat, 702; Mexico
A28 *S. somaliensis*, M. Mariat, 313
A29 *S. somaliensis*, M. Mariat, 314
A34 *S. somaliensis*, M. Mariat, 383
N20 *S. somaliensis*, NCTC 3236

(*e*) Strains assigned to cluster 5 (cluster 'B')

A44 '*Actinomadura*' sp., J. Lacey, A1248; hay
A45 '*Actinomadura*' sp., J. Lacey, A640; hay
A46 '*Actinomadura*' sp., J. Lacey, A336; hay
A53 '*Actinomadura*' sp., J. Lacey, A670; barley
A62 '*Actinomadura*' sp., J. Lacey, A66; straw
A63 '*Actinomadura*' sp., J. Lacey, A1276
A66 '*Actinomadura*' sp., J. Lacey, A694; straw
A71 '*Actinomadura*' sp., J. Lacey, A548; barley
A79 '*Actinomadura*' sp., J. Lacey, A1247; hay
A81 '*Actinomadura*' sp., J. Lacey, A637; barley
A85 '*Actinomadura*' sp., J. Lacey, A333; hay
A86 '*Actinomadura*' sp., J. Lacey, A320; barley
A89 '*Actinomadura*' sp., J. Lacey, A674; barley
N263 *Nocardia madurae*-type, T. Cross, Bradford University, CUB410; J. Lacey, stored hay

(*f*) Strains assigned to cluster 6 (cluster 'A')

A47 '*Actinomadura*' sp., J. Lacey, A676; bagasse
A48 '*Actinomadura*' sp., J. Lacey, A678; barley
A51 '*Actinomadura*' sp., J. Lacey, A337; oats
A54 '*Actinomadura*' sp., J. Lacey, A735; hay
A55 '*Actinomadura*' sp., J. Lacey, A428; barley
A64 '*Actinomadura*' sp., J. Lacey, A826; hay
A67 '*Actinomadura*' sp., J. Lacey, A491; barley
A68 '*Actinomadura*' sp., J. Lacey, A302; straw
A69 '*Actinomadura*' sp., J. Lacey, A307; barley
A70 '*Actinomadura*' sp., J. Lacey, A305; barley
A72 '*Actinomadura*' sp., J. Lacey, A410; barley
A73 '*Actinomadura*' sp., J. Lacey, A318; hay
A75 '*Actinomadura*' sp., J. Lacey, A673; barley
A77 '*Actinomadura*' sp., J. Lacey, A232; bagasse
[b]A80 '*Actinomadura*' sp., J. Lacey, A443; H. A. Lechevalier, Sal 1
A83 '*Actinomadura*' sp., J. Lacey, A581; barley
A84 '*Actinomadura*' sp., J. Lacey, A470; barley
A87 '*Actinomadura*' sp., J. Lacey, A1016; hay
A88 '*Actinomadura*' sp., J. Lacey, A824; bagasse
A90 '*Actinomadura*' sp., J. Lacey, A272; straw
A91 '*Actinomadura*' sp., J. Lacey, A620; hay
A104 '*Actinomadura*' sp., J. Lacey, A232; bagasse
A106 '*Actinomadura*' sp., J. Lacey, A630; air sample, barley silo

B. *Minor clusters*

(*g*) Strains assigned to cluster 7 (*Actinomadura roseoviolacea*)

[1]*A2 *Actinomadura roseoviolacea*, H. Nonomura, A-5; soil
[1]*A140 *A. roseoviolacea*, H. Nonomura, A-5; soil

(*h*) Strains assigned to cluster 8

A108 '*Actinomadura*' sp., J. Lacey, A1273; hay
A109 '*Actinomadura*' sp., J. Lacey, A1295; hay

Table 1 (*cont.*)

(*i*) Strains assigned to cluster 9 (*Rhodococcus* sp.)

A135 *Nocardia madurae*, Czechoslovak Collection of Microorganisms, J. E. Purkyne University, Brno, Czechoslovakia, CCM 5509
A136 *N. madurae*, CCM 5510

(*j*) Strains assigned to cluster 10 (*Streptomyces* sp.)

N205 *Nocardia gibsonii*, ATCC 6852, NCTC 4575; A. G. Gibson
N206 *Nocardia rangoonensis*, ATCC 6860, NCTC 1678; pulmonary streptothrichosis

(*k*) Strains assigned to cluster 11 (*Nocardia carnea*)

[j]N675 *Nocardia carnea*, R. E. Gordon, ATCC 6847
[j]N676 *N. carnea*, R. E. Gordon, 3419, ATCC 6847

(*l*) Strains assigned to cluster 12 (*Nocardia vaccinii*)

*N537 *Nocardia salmonicida*, T. G. Pridham, NRRL, Peoria, U.S.A.
[k]N681 *Nocardia vaccinii*, R. E. Gordon, 3500, ATCC 11092; N. R. Smith, BG19; stem galls, blueberry
[k]N682 *N. vaccinii*, R. E. Gordon, ATCC 11092

(*m*) Strains assigned to cluster 13 (*Nocardia transvalensis*)

[l]N679 *Nocardia transvalensis*, R. E. Gordon, 3426, NCTC 2392
[l]N680 *N. transvalensis*, R. E. Gordon, NCTC 2392

C. Clusters containing single strains

*A5 *Actinomadura helvata*, H. Nonomura, A-105; soil
A6 *Actinomadura madurae*, C. Philpot, 391
A127 *A. madurae*, H. Prauser, IMET 9562; soil, Japan
*A3 *Actinomadura pusilla*, H. Nonomura, A-118
*A1 *Actinomadura spadix*, H. Nonomura, A-116
A57 '*Actinomadura*' sp., J. Lacey, A879; coffee beans
A61 '*Actinomadura*' sp., J. Lacey, A893; bagasse
A76 '*Actinomadura*' sp., J. Lacey, A440; air
A82 '*Actinomadura*' sp., J. Lacey, A429; barley
N609 *Micropolyspora caesia*, J. Lacey, A1194
*N673 *Nocardia aerocolonigenes*, R. E. Gordon, ISP 5034
*N317 *Nocardia asteroides*, ATCC 19247; R. E. Gordon, 727
*N318 *Nocardia brasiliensis*, ATCC 19296; R. E. Gordon, 845
N215 *Nocardia formica*, J. E. Thiemann, Lepetit SpA, Milan, Italy, S542
N614 *Nocardia fukuyae*, S. Suzuki, Rikagaku, Kenkyusho, Wako-Shi, Saitano, Japan, 11/41
N68 *Nocardia gardneri*, Institute of Applied Microbiology, University of Tokyo, Tokyo, IAM0105
N531 *Nocardia italica*, CBS 609.67
N323 *Nocardia keratolytica*, ATCC 12484
A94 *Nocardia madurae*, W. A. Causey, CDC N273; Dr Londero, Brazil; burn eschar
A110 *N. madurae*, W. A. Causey, CDC N248; sputum
A111 *N. madurae*, W. A. Causey, CDC N272
A137 *N. madurae*, laboratory strain, K657
N319 *Nocardia saturnea*, ATCC 15778; P. Hirsch, 99; compost
A98 *Nocardia* (*Proactinomyces*) *gardneri*, V. D. Kuznetsov, RIA 634
A103 *Nocardia* (*Proactinomyces*) *madurae*, V. D. Kuznetsov, 452
A102 *Nocardia* (*Proactinomyces*) *polychromogenes*, V. D. Kuznetsov, RIA 675
A99 *Nocardia* (*Proactinomyces*) *tenuis*, V. D. Kuznetsov, RIA 638
N412 *Streptosporangium* sp., laboratory strain
N497 *Streptosporangium* sp., laboratory strain

* Type strains.
† Superior letters preceding laboratory numbers indicate triplicate (a, c) or duplicate (b, d to l) cultures.

computer using both the simple matching coefficient (S_{SM}; Sokal & Michener, 1958), which includes both positive and negative matches, and the Jaccard coefficient (S_J; Sneath, 1957) which includes positive matches only. Clustering was achieved using both single linkage and unweighted average linkage (UPGMA) algorithms (Sneath & Sokal, 1974). Vigour and pattern statistics were also calculated (Wishart, 1968) and a sorted shaded diagram was prepared from the product of the pattern coefficient (D_P), UPGMA analysis.

Analysis for mycolic acids. Three '*Actinomadura*' cultures (A46, A84, A88) and two cultures received as *Nocardia madurae* (A135, A136) were examined for mycolic acids using the medium and methods described by Minnikin *et al.* (1975).

RESULTS

Clustering of the strains using the D_P coefficient with the UPGMA algorithm

The percentage dissimilarity (D_T) between strains can be divided into vigour (D_V) and pattern (D_P) components (Sneath, 1968). D_V expresses differences in total metabolic activity between strains, as measured by numbers of positive reactions, while D_P takes account of which tests give positive results, allowing for differences in growth rates, periods of incubation and similar factors which normally distort similarity values. Since the test strains varied widely in rates of growth, classification based on the pattern coefficient, UPGMA analysis is described in detail.

Of the 156 test cultures, 137 were recovered in six major and seven minor clusters (Fig. 1). All clusters were distinct and homogeneous and were named, where possible, after type or authentic named strains found within them (Table 1). The properties of the major and minor clusters are given in Tables 2 and 3, respectively.

Most of the 23 strains in cluster 1 were isolated from clinical sources and identified as *Actinomadura* (or *Nocardia* or *Streptomyces*) *madurae* (Table 1). All three cultures of the type strain of *A. madurae* (A16, A113, A142) were recovered near the centre of this cluster but duplicate cultures of another strain, A125 and A80, were found, respectively, in clusters 1 and 6 (Table 1). Cluster 1 also included two isolates (A74, A107) from hay and barley, respectively, and the type cultures of *Actinomadura malachitica* (A139), *Actinomadura citrea* (A138) and *Actinomadura verrucosospora* (A4). It may be significant that these three showed a greater similarity to one another than to other strains in the cluster. Most of the remaining cultures received as *A.* (or *N.*) *madurae* (A6, A94, A110, A111, A127, A137) showed little similarity to cultures in clusters 1 or 6 or to one another, but two (A135, A136) formed cluster 9. The two strains in this cluster produced red colonies, a primary mycelium that soon fragmented into irregular elements, contained mycolic acids, grew on a wide range of carbon sources and were sensitive to lysozyme (Table 3). They thus resemble species of *Rhodococcus* (Goodfellow & Alderson, 1977) and have probably been misidentified.

Cluster 2 contained 14 isolates from fodder, soil and clinical material and included duplicates of the type strain of *Actinomadura* (or *Nocardia*) *dassonvillei* (A14, A118). All but one of the isolates were received as *A.* (or *N.*) *dassonvillei*, the exception (A49) being labelled '*Actinomadura*' sp.

The 31 strains forming the largest cluster, cluster 3, showed some heterogeneity although all were received as *Actinomadura* (or *Nocardia* or *Streptomyces*) *pelletieri* (Fig. 1), with one exception (A132) labelled *A. madurae*. Duplicates of the type culture of *A.* (or *N.*) *pelletieri* (A19, A143) were both found in cluster 3. The cluster was distinct from cluster 4 which contained six strains of *Streptomyces somaliensis* and one evidently misidentified as *N. pelletieri* (A18).

Most isolates from grain and fodder were recovered in clusters 5 and 6, labelled clusters 'B' and 'A', respectively. Cluster 6 contained 23 isolates. Cluster 5 contained 14, one of which (N263) had previously been recovered by Goodfellow (1971) within subgroup A of his *N. madurae* group. Other '*Actinomadura*' isolates were either recovered in cluster 8 (A108, A109) or as single strains (A57, A61, A76, A82). Cluster 7 contained only the duplicate cultures of *Actinomadura roseoviolacea* (A2, A140). The type cultures of *Actinomadura helvata* (A5), *Actinomadura pusilla* (A3) and *Actinomadura spadix* (A1) were not recovered in any of the defined clusters although the last was similar to *A. roseoviolacea* strains.

Marker cultures of *Nocardia*, *Micropolyspora* and *Streptosporangium* showed little similarity to the *Actinomadura* cultures, to the bagasse and fodder isolates or to one another (Fig. 1). Cluster 10 was quite distinct and contained strains received as *Nocardia gibsonii* (N205) and *Nocardia rangoonensis* (N206). The walls of both species contain LL-diaminopimelic acid and lack characteristic sugars (G. H. Bowden, personal communication) so

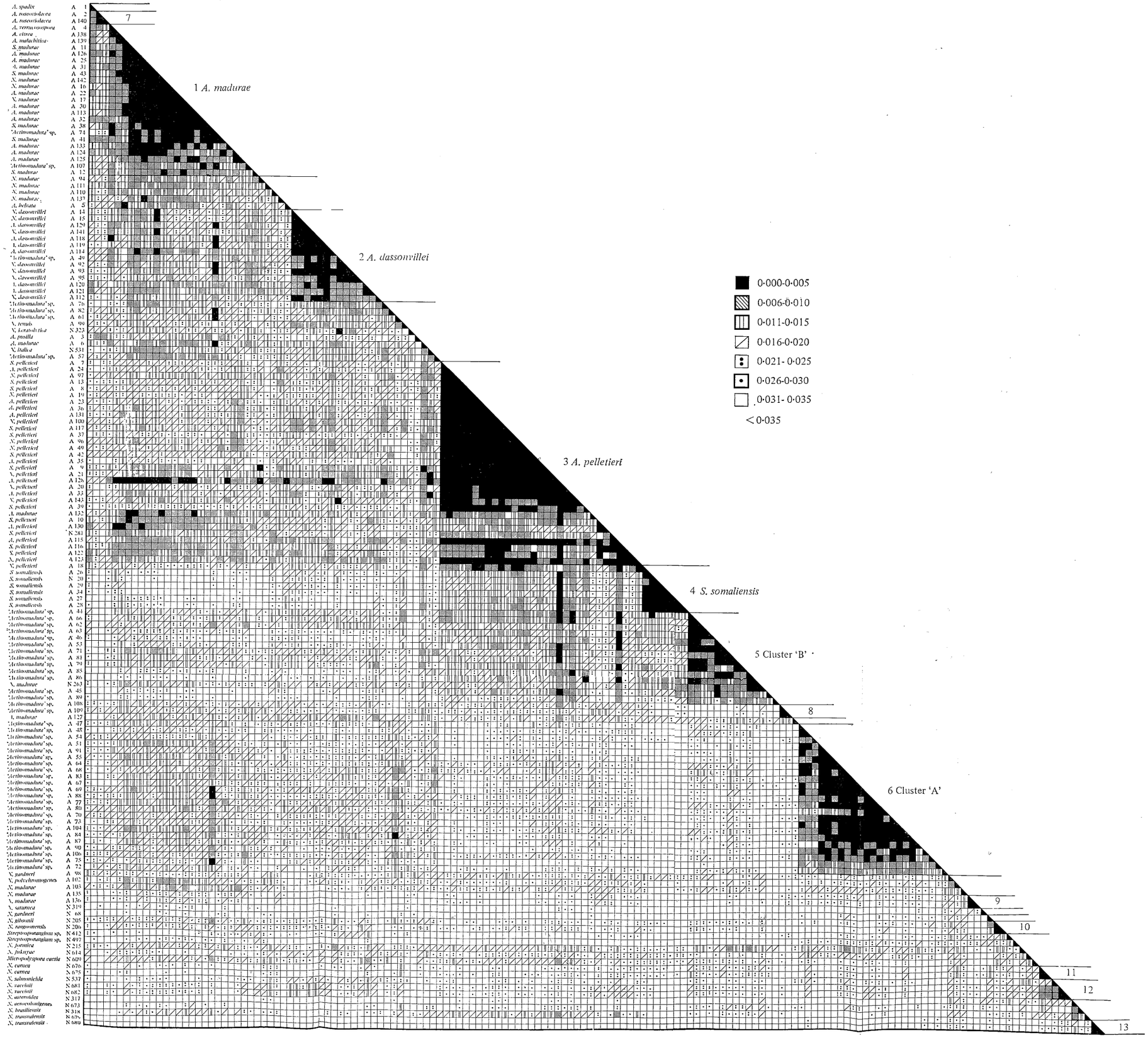

Fig. 1. Pattern matrix of the 156 test cultures based on 90 unit characters.

that their classification in the genus *Streptomyces* seems appropriate. Clusters 11 and 13 contained duplicate cultures of *Nocardia carnea* (N675, N676) and *N. transvalensis* (N679, N680), respectively. Cluster 12 included the duplicate cultures of *N. vaccinii* (N681, N682) with *N. salmonicida* (N537) loosely attached.

Clustering of the strains using the S_{SM} and S_J coefficients with the UPGMA algorithm

Cluster composition was only marginally affected when the S_J and S_{SM} coefficients were used with the UPGMA algorithm but the clusters were arranged differently (Figs 2 and 3). In each analysis, cluster 'B' and the *S. somaliensis* phenon were recovered in their entirety, as were the minor clusters with the exception of cluster 12 from which *N. salmonicida* (N537) was excluded.

Both the S_J and S_{SM} analyses modified cluster 1 (*A. madurae*) by excluding *A. verrucosospora* A4 and the isolates A74 and A107. These three cultures remained differentiated from all of the defined clusters. S_J analysis also separated *A. madurae* A132 from cluster 3 (*A. pelletieri*) and A80, the duplicate of A125, from cluster 6 (cluster 'B'). S_{SM} analysis grouped A132 with cluster 1 (*A. madurae*) and separated A80 from cluster 6. It also removed five isolates (A10, A116, A122, A130, N281), which formed a loose subcluster by D_P analysis, from cluster 3 to form a new cluster, 14 (Fig. 2).

Major clusters were recovered in two aggregates by S_{SM} analysis (Fig. 2). One contained clusters 1 (*A. madurae*), 2 (*A. dassonvillei*), 6 (cluster 'A'), and 14 (*A. pelletieri*); the other included clusters 3 (*A. pelletieri*), 4 (*S. somaliensis*) and 5 (cluster 'B'). The first of these aggregates was also recovered by S_J analysis, but with this coefficient clusters 3, 4 and 5 showed little similarity to one another or to any other major cluster (Fig. 3). In the corresponding D_P analysis, two aggregates were formed; the first contained *A. dassonvillei* and *A. madurae*, the second *A. pelletieri*, *S. somaliensis* and cluster 'B'. Cluster 'A' shared little similarity with either (Fig. 1). Only *A. dassonvillei* and *A. madurae* were consistently recovered in the same aggregate of clusters, but even they were only loosely associated in the S_J analysis.

Clustering of the strains using the S_{SM} and S_J coefficients with the single linkage algorithm

The classifications obtained with the single linkage clustering method were almost the same as those formed in the UPGMA analyses. However, cluster 14 was recovered in both the S_J and S_{SM} analyses, while in the S_{SM} analysis the *A. pelletieri* and *S. somaliensis* clusters formed two subgroups within a common cluster. Also strains A74 and A107 were recovered in cluster 6 (cluster 'A'); otherwise, cluster composition was not affected.

Reproducibility of results

Inclusion of duplicate and triplicate strains (Table 1) in the analysis enabled experimental test error to be estimated. The probability (p) of an erroneous result averaged 4·5 %, equal to an observed S_{SM} value of about 91 % between duplicate cultures.

Characters useful for the separation of Actinomadura species and allied taxa

Characters potentially useful for the differentiation of *Actinomadura* species and related taxa are shown in Table 4.

Analysis for mycolic acids

Mycolic acids were detected in two cultures received as *Nocardia madurae* (A135, A136) but not in the representatives of clusters 'A' (A84, A88) and 'B' (A46).

Table 2. *Percentage frequencies of positive characters found in the major clusters*

	Actinomadura madurae	*Actinomadura dassonvillei*	*Actinomadura pelletieri*	*Streptomyces somaliensis*	Cluster 'B'	Cluster 'A'
Cluster no.	1	2	3	4	5	6
No. of strains	23	14	31	7	14	23
Colonial characters:						
Aerial hyphae sparse	26	86	30	72	36	79
Aerial hyphae moderate	18	86	3	57	29	75
Aerial hyphae abundant	13	72	0	29	0	44
Spines/tufts formed	4	57	3	43	7	9
Margin filamentous	88	100	68	100	86	96
Margin deeply filamentous	9	79	0	72	0	26
Elevation convex	26	7	19	0	7	9
Elevation crateriform	22	7	10	100	22	35
Elevation flat/raised	4	29	0	0	0	4
Elevation irregular	62	50	71	14	86	62
Colonies cream/white	79	86	23	86	86	88
Colonies pink/orange	4	7	7	0	0	9
Colonies red/maroon	13	0	68	14	0	0
Degradation of:						
Adenine	9	86	0	0	29	88
Aesculin	100	100	3	0	57	100
Casein	100	100	100	100	100	9
Chitin	9	43	74	0	7	84
DNA	70	100	16	100	29	66
Elastin	100	100	100	100	72	0
Gelatin	100	100	100	100	100	91
Guanine	4	93	0	0	0	100
Hypoxanthine	100	100	90	0	93	100
Keratin	100	100	100	100	100	0
RNA	96	100	7	100	93	100
Starch	65	93	0	0	100	95
Testosterone	96	7	13	72	7	100
Tyrosine	100	100	100	100	93	100
Urea	9	0	0	0	0	0
Xanthine	9	100	0	0	86	91
Growth on sole carbon source (1 %, w/v):						
Adonitol	96	22	3	0	86	100
D-Arabinose	9	0	0	0	29	88
L-Arabinose	100	93	13	0	7	100
Amygdalin	0	22	0	0	0	0
Arbutin	0	7	0	0	7	9
Cellobiose	96	100	16	0	22	88
Dextrin	100	100	19	0	7	100
Dulcitol	0	0	0	0	7	0
Erythritol	26	7	0	0	86	100
Ethanol	66	0	3	0	29	96
Fructose	100	86	16	0	14	100
Galactose	88	100	16	0	93	100
Glycerol	100	100	19	0	100	100
Glycogen	88	100	19	0	7	96
Inositol	44	0	0	0	0	96
Inulin	4	7	0	0	0	13
Lactose	9	43	0	0	14	9
Maltose	100	100	68	72	7	100
Mannitol	100	100	19	0	100	96
Mannose	88	86	3	0	50	100
Melibiose	9	43	0	0	0	70
α-Methyl-D-glucoside	9	7	0	0	22	18

Table 2 (*cont.*)

	Actinomadura madurae	*Actinomadura dassonvillei*	*Actinomadura pelletieri*	*Streptomyces somaliensis*	Cluster 'B'	Cluster 'A'
β-Methyl-D-glucoside	22	100	3	0	29	65
Raffinose	9	27	0	0	0	4
Rhamnose	100	43	19	0	72	100
Salicin	9	72	0	0	7	18
Sorbitol	0	0	0	0	0	9
Starch	96	100	19	0	29	96
Sucrose	88	100	29	14	22	96
Trehalose	100	100	84	0	22	91
Xylose	100	86	13	0	0	9
Growth on sole carbon source (0·1 %, w/v):						
Glucosamine	57	29	13	0	7	91
m-Hydroxybenzoic acid	4	0	45	0	0	57
Sodium acetate	62	43	23	0	29	96
Sodium adipate	4	7	0	0	7	57
Sodium butyrate	91	93	90	14	93	91
Sodium octanoate	22	72	3	0	29	9
Sodium propionate	65	72	42	0	93	44
Sodium tartrate	0	0	0	0	7	4
Resistance to:*						
Demethylchlortetracycline (500)	40	29	36	100	100	57
Cephaloridine (100)	0	7	19	86	36	26
Gentamicin (100)	35	100	87	100	100	70
Lincomycin (100)	0	0	10	86	57	4
Neomycin (50)	88	93	48	100	93	4
Oleandomycin (100)	0	0	0	43	64	0
Penicillin (10 i.u.)	9	0	90	100	86	88
Rifampicin (50)	0	64	0	86	93	31
Streptomycin (100)	65	43	19	86	86	75
Tobramycin (50)	79	100	58	100	100	9
Vancomycin (50)	31	43	32	86	100	79

* Filter paper discs were soaked in antimicrobial agent at the concentrations (μg ml^{-1}) given in parentheses; for penicillin, Oxoid discs were used.

No strains degraded xylan, produced allantoinases or brown colonies, were resistant to lysozyme or were able to use melezitose (1 %, w/v) or testosterone (0·1 %, w/v) as sole carbon sources. All strains degraded Tweens 20, 40, 60 and 80 and used glucose (1 %, w/v) as a sole carbon source.

DISCUSSION

The numerical analyses confirm the sharp separation of *Actinomadura* and *Nocardia* (Tsukamura, 1969; Goodfellow, 1971). They also show that *A. dassonvillei*, *A. madurae*, *S. somaliensis* and clusters 'A' and 'B' form good taxospecies, distinguishable by many characters (Table 4). However, the separation of *A. pelletieri* strains into two clusters by S_J and S_{SM} analyses requires further study. The affinities of representatives of new *Actinomadura* species are still unclear, although *A. helvata*, *A. pusilla*, *A. roseoviolacea*, *A. spadix* and *A. verrucosospora* (Nonomura & Ohara, 1971) may form new centres of variation. However, *A. citrea* and *A. malachitica* (Lavrova *et al.*, 1972) show some similarity with *A. verrucosospora* as well as with *A. madurae*; and *A. spadix* is close to *A. roseoviolacea*. However, further comparison of the new species using more isolates, if possible, is required. Pattern differences between species based on single isolates may be unreliable as they cannot take account of the range of variation that may be present in a species.

Similarity values in numerical phenetic surveys can be influenced by differences in growth rates, cultural conditions, test error, lack of test reproducibility, sampling error and by

Table 3. *Distribution of positive characters to the minor clusters and amongst the representatives of the Actinomadura species which were not recovered in major or minor clusters*

	A. helvata	*A. pusilla*	*A. roseoviolacea*	*A. spadix*	*A. verrucosospora*	'*Actinomadura*' sp.	*Rhodococcus* sp.	*Streptomyces* sp.	*N. carnea*	*N. transvalensis*	*N. vaccinii*
Cluster no.	—	—	7	—	—	8	9	10	11	13	12
No. of strains	1	1	2	1	1	2	2	2	2	2	2*
Colonial characters:											
Aerial hyphae sparse	1	1	2	1	1	0	2	2	2	2	2
Aerial hyphae moderate	0	1	1	1	1	0	0	2	2	2	2
Aerial hyphae abundant	0	0	0	0	0	0	0	2	0	2	2
Spines/tufts formed	0	0	2	1	1	0	0	0	0	2	2
Margin filamentous	0	1	2	1	0	1	0	2	2	0	2
Margin deeply filamentous	0	1	0	0	0	0	0	1	0	0	0
Elevation convex	0	0	0	1	0	0	2	1	0	2	0
Elevation crateriform	0	1	2	0	1	2	0	1	0	0	0
Elevation flat/raised	0	0	0	0	0	0	0	0	0	0	0
Elevation irregular	1	0	0	0	0	1	0	0	2	0	2
Colonies cream/white	1	0	0	0	1	0	0	2	0	0	0
Colonies pink/orange	0	0	2	0	0	0	0	0	2	0	2
Colonies red/maroon	0	0	0	0	0	2	2	0	0	2	0
Colonies brown	0	1	0	1	0	0	0	0	0	0	0
Degradation of:											
Adenine	0	0	0	0	0	0	0	0	0	0	0
Aesculin	0	1	0	0	1	0	0	1	2	2	2
Allantoin	0	0	0	0	0	1	0	2	0	2	0
Casein	1	1	2	1	1	2	0	2	0	0	0
Chitin	0	0	0	0	0	1	0	0	0	0	0
DNA	1	1	2	1	1	2	0	2	0	0	0
Elastin	1	0	0	0	1	2	0	1	0	0	0
Gelatin	1	1	2	1	1	2	0	2	0	0	0
Guanine	0	0	0	0	0	0	0	0	0	0	0
Hypoxanthine	0	1	2	0	1	0	0	2	0	2	0
Keratin	1	1	2	1	1	2	0	0	0	0	0
RNA	1	1	2	1	1	2	2	0	2	0	0
Starch	0	0	0	1	0	1	0	0	0	0	0
Testosterone	1	1	2	1	1	1	2	2	2	0	0
Tween 20	1	1	2	1	1	2	2	2	0	2	0
Tween 40	1	1	2	1	1	2	2	2	0	0	0
Tween 60	1	1	2	1	1	2	2	2	0	1	0
Tween 80	1	1	2	1	1	2	2	2	0	0	0
Tyrosine	0	1	2	0	1	0	2	0	0	2	0
Urea	0	0	0	0	0	0	0	2	0	2	2
Xanthine	0	0	0	0	0	0	0	2	0	0	0
Xylan	0	0	0	0	0	2	0	0	0	0	0
Growth on sole carbon source (1 %, w/v):											
Adonitol	0	1	2	1	1	0	2	2	0	2	0
D-Arabinose	0	0	0	0	0	0	0	0	0	2	0
L-Arabinose	1	1	2	1	1	2	2	0	0	0	2
Amygdalin	0	0	0	0	0	0	0	2	0	0	0
Arbutin	0	0	0	0	0	0	2	0	0	0	0
Cellobiose	1	1	2	1	1	2	1	2	0	0	0
Dextrin	0	1	2	1	1	2	2	2	0	0	2
Dulcitol	0	0	0	0	0	0	0	0	0	0	0
Erythritol	0	1	0	0	0	0	2	2	0	1	0
Ethanol	0	1	1	0	0	0	0	1	0	2	0
Fructose	1	1	2	1	1	2	2	2	0	2	0
Galactose	0	1	2	1	1	2	2	2	2	2	2

Table 3 (*cont.*)

	A. helvata	*A. pusilla*	*A. roseoviolacea*	*A. spadix*	*A. verrucosospora*	'*Actinomadura*' sp.	*Rhodococcus* sp.	*Streptomyces* sp.	*N. carnea*	*N. transvalensis*	*N. vaccinii*
Glucose	1	1	2	1	1	2	2	2	2	2	2
Glycerol	0	1	2	1	1	2	2	2	2	2	2
Glycogen	0	0	2	1	1	2	2	0	0	0	2
Inositol	1	0	0	0	0	0	2	1	0	2	0
Inulin	0	1	2	0	0	1	0	2	0	1	0
Lactose	0	0	2	1	0	1	0	2	0	1	0
Maltose	1	1	2	1	1	2	2	2	0	0	0
Mannitol	0	0	0	1	1	0	0	2	2	2	2
Mannose	1	1	2	0	0	2	2	2	0	2	0
Melezitose	0	1	1	0	0	0	2	0	0	0	0
Melibiose	0	0	0	0	0	2	0	0	0	0	0
α-Methyl-D-glucoside	0	0	0	0	0	0	0	2	0	0	0
β-Methyl-D-glucoside	0	1	0	0	0	2	1	1	0	0	0
Raffinose	0	0	2	1	0	2	0	0	0	0	0
Rhamnose	0	1	2	1	1	1	2	0	0	0	2
Salicin	1	1	2	0	0	0	0	1	0	0	0
Sorbitol	0	0	0	0	0	0	2	0	0	2	0
Starch	0	1	2	1	1	2	2	0	0	0	0
Sucrose	1	1	2	1	1	2	2	2	0	2	0
Trehalose	0	1	2	1	1	2	2	2	2	0	0
Xylose	1	1	2	1	1	2	2	2	0	0	0
Growth on sole carbon source (0·1 %, w/v):											
Glucosamine	0	1	2	1	0	2	2	0	0	0	0
m-Hydroxybenzoic acid	0	1	0	0	0	0	2	0	0	0	0
Sodium acetate	0	1	2	1	0	0	2	2	2	2	0
Sodium adipate	0	0	0	0	0	0	0	0	0	2	0
Sodium butyrate	0	1	2	1	1	0	2	2	2	2	0
Sodium octanoate	0	0	0	0	1	0	0	0	0	2	0
Sodium propionate	0	1	0	0	1	2	2	2	2	2	2
Sodium tartrate	0	0	0	0	0	0	0	0	0	0	0
Testosterone	0	0	0	0	0	0	0	0	2	2	0
Resistance to:†											
Demethylchlortetracycline (500)	1	0	1	1	0	2	2	2	0	0	0
Cephaloridine (100)	0	0	0	0	0	0	0	0	2	0	0
Gentamicin (100)	0	0	0	1	0	2	2	2	2	0	2
Lincomycin (100)	0	0	0	0	0	0	2	0	0	0	0
Neomycin (50)	0	0	0	1	1	2	2	2	2	0	2
Oleandomycin (100)	0	0	0	0	0	0	0	0	0	0	0
Penicillin (10 i.u.)	1	0	0	0	0	2	2	0	1	0	0
Rifampicin (50)	0	0	0	0	0	0	2	0	0	0	0
Streptomycin (100)	1	0	0	1	0	2	2	2	2	0	2
Tobramycin (50)	0	1	1	1	0	0	2	2	2	0	2
Vancomycin (50)	1	0	0	1	0	2	2	2	2	0	0
Resistance to:											
Lysozyme	1	1	2	1	0	2	2	2	2	0	2

* *Nocardia salmonicida* omitted.

† See Table 2.

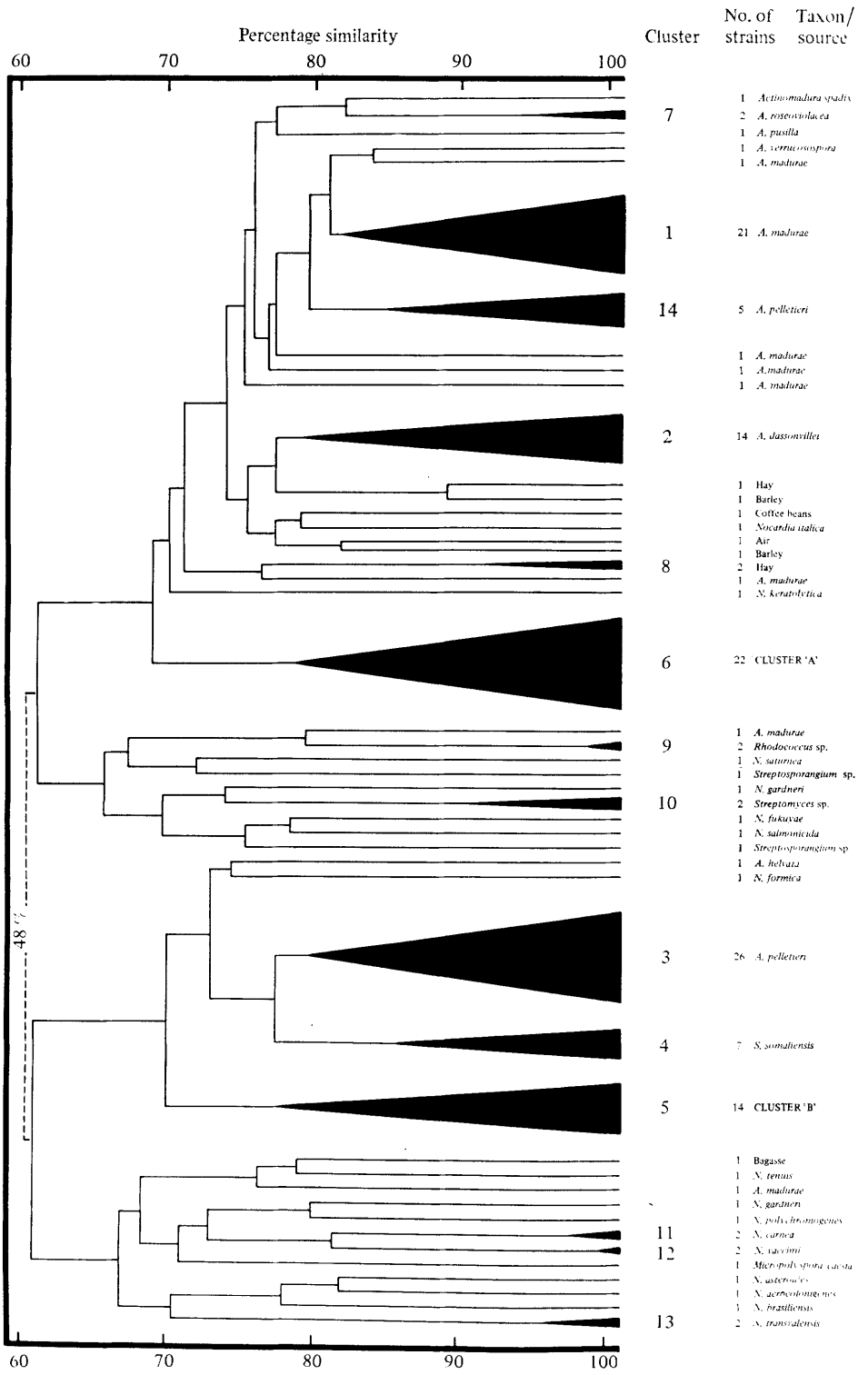

Fig. 2. A simplified dendrogram showing the relationships between clusters based on the S_{SM} coefficient and unweighted average linkage clustering (UPGMA).

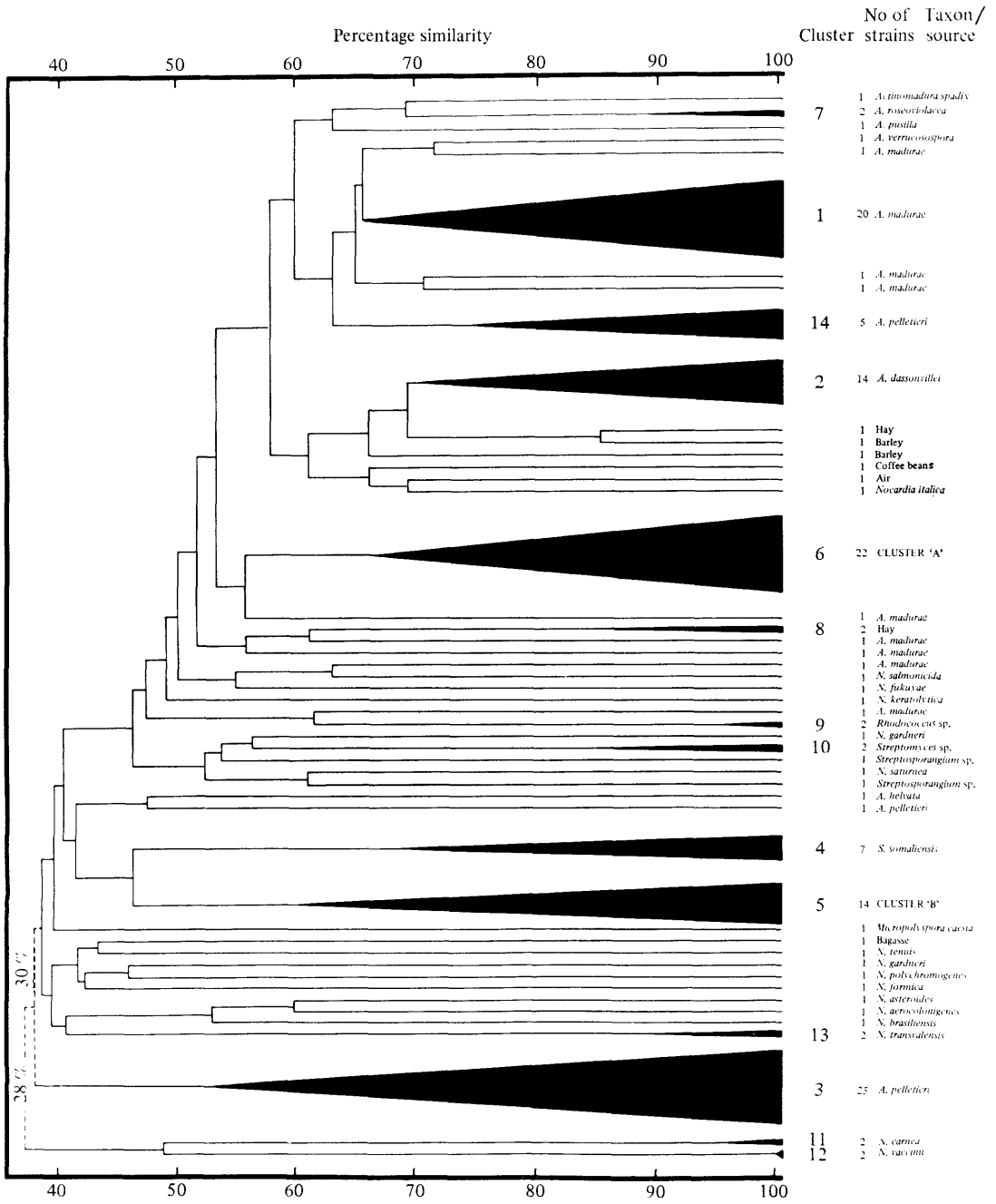

Fig. 3. A simplified dendrogram showing the relationships between clusters based on the S_J coefficient and unweighted average linkage clustering (UPGMA).

Table 4. *Distinguishing characters for Actinomadura species and allied taxa*

	A. dassonvillei	*A. helvata*	*A. madurae*	*A. pelletieri*	*A. pusilla*	*A. roseoviolacea*	*A. spadix*	*A. verrucosospora*	Cluster 'A'	Cluster 'B'	*S. somaliensis*
Cluster no.	2	—	1	3	—	7	—	—	6	5	4
No. of strains	14	1	23	31	1	2	1	1	23	14	7
Degradation of:											
Adenine	+	−	−	−	−	−	−	−	+	d	−
Aesculin	+	−	+	−	+	−	−	+	+	d	−
Casein	+	+	+	+	+	+	+	+	−	+	+
Elastin	+	+	+	+	−	−	−	+	−	d	+
Guanine	+	−	−	−	−	−	−	−	+	−	−
Hypoxanthine	+	−	+	+	+	+	−	+	+	+	−
Keratin	+	+	+	+	+	+	+	+	−	+	+
RNA	+	+	+	−	+	+	+	+	+	+	+
Starch	+	−	d	−	−	−	+	−	+	+	−
Testosterone	−	+	+	−	+	+	+	+	−	−	d
Tyrosine	+	−	+	+	+	+	−	+	+	+	+
Xanthine	+	−	−	−	−	−	−	−	+	+	−
Sole carbon source (at 1 %, w/v):											
Adonitol	d	−	+	−	+	+	+	+	+	+	−
D-Arabinose	−	−	−	−	−	−	−	−	+	d	−
L-Arabinose	+	+	+	−	+	+	+	+	+	−	−
Cellobiose	+	+	+	−	+	+	+	+	+	d	−
Dextrin	+	−	+	d	+	+	+	+	+	−	−
Erythritol	−	−	d	−	+	−	−	−	+	+	−
Ethanol	−	−	d	−	+	d	−	−	+	d	−
Fructose	+	+	+	−	+	+	+	+	+	−	−
Galactose	+	−	+	d	+	+	+	+	+	+	−
Glycerol	+	−	+	d	+	+	+	+	+	+	−
Glycogen	+	−	+	d	−	+	+	+	+	−	−
Inositol	−	+	d	−	−	−	−	−	+	−	−
Maltose	+	+	+	d	+	+	+	+	+	−	d
Mannitol	+	−	+	d	−	−	+	+	+	+	−
Mannose	+	+	+	−	+	+	−	−	+	d	−
Rhamnose	d	−	+	d	+	+	+	+	+	d	−
Trehalose	+	−	+	+	+	+	+	+	+	d	−
Xylose	+	+	+	−	+	+	+	+	−	−	−
Sole carbon source (at 0·1 %, w/v):											
Sodium butyrate	+	−	+	+	+	+	+	+	+	+	−
Resistance to:											
Penicillin (10 i.u.)	−	+	−	+	−	−	−	−	+	+	+

+, More than 85 % of strains positive; −, more than 85 % of strains negative; d, strains 16 to 84 % positive.

the coefficients and clustering algorithms employed (Sneath, 1968; Sneath & Johnson, 1972; Austin & Colwell, 1977; Goodfellow, 1977). Thus, the unexpectedly large similarities reported between *A. pelletieri* and *Nocardia sensu stricto* (Holmberg & Hallander, 1973), between *Actinomadura* and *S. somaliensis* (Kurup & Schmitt, 1973) and between *A. pelletieri* and *Rothia dentocariosa* (Jones, 1975) can probably be accounted for by such factors. Such spurious similarities and dissimilarities can be detected by evaluating numerical data with different coefficients of association and clustering algorithms and comparing results with classification based on other types of data, e.g. chemical, genetical and morphological (Goodfellow *et al.*, 1978).

Many different coefficients have been used successfully in numerical taxonomic studies of bacteria, but their choice tends to be subjective and comparisons are few (Austin & Colwell 1977). The significance of negative matches and possible distortions due to growth rate must be taken into account. The Jaccard coefficient (S_J; Sneath, 1957) excludes negative matches, thus emphasizing differences between strains with few positive characters in common. Many of our strains showed few positive characters and differed markedly in growth rate. For this reason, data were analysed using S_{SM} and D_P coefficients as well as S_J. The use of different clustering methods does not affect the formation of clusters where these are homogeneous and there are no intermediates. However, where there are intermediates, average linkage gives much more compact clusters than single linkage.

Our classification has been primarily based on pattern differences although cluster composition was not markedly affected either by the coefficients or clustering algorithms used or by the 4·5% test error. However, the coefficients of association used did affect the relationships between the major clusters while the similarity between *A. pelletieri* and *S. somaliensis* in S_{SM} analyses owed much to negative correlation (Figs 1, 2 and 3). These numerical findings are difficult to interpret. While the major clusters, with the exception of cluster 14, can be considered to represent good taxospecies, little can be said about the grouping of these species into genera. At present there is no objective way of equating similarity values with orthodox taxonomic rank, but numerical data can be interpreted in the light of results collected using independent taxonomic criteria.

Chemical characters have been particularly useful in evaluating numerical phenetic classifications (Lechevalier & Lechevalier, 1970; Lacey & Goodfellow, 1975; Goodfellow & Minnikin, 1978). Thus, *S. somaliensis*, like other streptomycetes, has a wall chemotype I (Becker *et al.*, 1965; Pridham & Lyons, 1969) and can thereby be distinguished from *Actinomadura* with a type III wall. These two taxa can also be distinguished by polar lipid composition (Lechevalier *et al.*, 1977), isoprenoid quinone content (Collins *et al.*, 1977) and data from serological analyses (González-Ochoa & Vásquez-Hoyos, 1953; Schneidau & Shaffer, 1957). The chemical and serological data in conjunction with the numerical data from the S_J analysis provide good grounds for continuing to classify *S. somaliensis* and *A. pelletieri* strains in separate genera.

Although the strains in clusters 'A' and 'B' have a morphology resembling *Actinomadura*, they showed little similarity with the phena representing the original three species of this genus. The sole exceptions (A74 and A107) within cluster 1 (*A. madurae*) were, in fact, removed from this cluster by S_J and S_{SM} analyses. These strains were isolated from hay and the air of a barley silo, respectively, and may be misplaced from cluster 6 (cluster 'A'). Clusters 'A' and 'B' were also clearly distinguished from the *Nocardia* clusters and from the type strains of *N. asteroides* and *N. brasiliensis*. Preliminary chemical data support the separation of clusters 'A' and 'B' strains from both *Nocardia* and *Actinomadura*. Representative strains of these taxa contain *meso*-diaminopimelic acid, arabinose and galactose (G. H. Bowden, personal communication) but, unlike nocardiae, do not contain mycolic acids. The relationships of the isolates provisionally classified in clusters 'A' and 'B' are in need of systematic study with actinomycetes having similar chemical and morphological features. Chemical criteria should not necessarily outweigh other taxonomic criteria although they have helped improve the classification of actinomycetes (Lechevalier *et al.*, 1977; Goodfellow & Minnikin, 1978).

The new genus *Nocardiopsis* has been proposed for isolates previously classified as *A. dassonvillei* (Meyer, 1976). This proposal has much to commend it, as *A. dassonvillei* differs from *A. madurae* and *A. pelletieri* in many characteristics (Lacey *et al.*, 1978), but our study raises some doubts about its advisability at present. While cluster 2 (*A. dassonvillei*) shares little similarity with clusters 1 (*A. madurae*) and 3 (*A. pelletieri*) the latter are even more sharply distinguished. Tsukamura (1969) also found little similarity between *A. madurae* and *A. pelletieri* strains. Since *A. madurae* and *A. pelletieri* show many chemical

similarities, further systematic studies are required to establish the degree of their relationship.

Two ways of treating the genus *Actinomadura* as constituted by Lechevalier & Lechevalier (1970) thus seem possible at present. Either the original concept can be retained or the removal of *A. dassonvillei* to *Nocardiopsis* can be accepted. In the latter case there would be equal justification on phenetic grounds for creating a new genus for strains of *A. pelletieri* and possibly dividing it into two species. Given the difficulties associated with the classification of *Actinomadura* and the lack of systematic studies of the newly described species, the more cautious alternative seems preferable at present.

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REFERENCES

AGRE, N. S., EFIMOVA, T. P. & GUZEVA, L. N. (1975). Heterogeneity of the genus *Actinomadura* Lechevalier and Lechevalier. *Microbiologiya* **44**, 252–257.

AUSTIN, B. & COLWELL, R. R. (1977). Evaluation of some coefficients for use in numerical taxonomy of micro-organisms. *International Journal of Systematic Bacteriology* **27**, 204–210.

BECKER, B., LECHEVALIER, M. P. & LECHEVALIER, H. A. (1965). Chemical composition of cell-wall preparations from strains of various form-genera of aerobic actinomycetes. *Applied Microbiology* **13**, 236–243.

BROCQ-ROUSSEU, M. D. (1904). Sur un *Streptothrix* cause de l'altération des avoines moisies. *Revue générale de botanique* **16**, 219–230.

COLLINS, M. D., PIROUZ, T., GOODFELLOW, M. & MINNIKIN, D. E. (1977). Distribution of menaquinones in actinomycetes and corynebacteria. *Journal of General Microbiology* **100**, 221–230.

CROSS, T. & GOODFELLOW, M. (1973). Taxonomy and classification of the actinomycetes. In *Actinomycetales: Characteristics and Practical Importance*, pp. 11–112. Edited by G. Sykes & F. A. Skinner. London: Academic Press.

GONZÁLEZ-OCHOA, A. & VÁSQUEZ-HOYOS, A. (1953). Relationes serogiaes de los principales actinomycetes patogens. *Revista del Instituto de salubridad y enfermedades tropicales, Mexico* **13**, 177–187.

GOODFELLOW, M. (1971). Numerical taxonomy of some nocardioform bacteria. *Journal of General Microbiology* **69**, 33–80.

GOODFELLOW, M. (1977). Numerical taxonomy. In *CRC Handbook of Microbiology*, 2nd edn, vol. 1. *Bacteria*, pp. 579–596. Edited by A. I. Laskin & H. A. Lechevalier. Ohio: CRC Press.

GOODFELLOW, M. & ALDERSON, G. (1977). The actinomycete-genus *Rhodococcus*: a home for the '*rhodochrous*' complex. *Journal of General Microbiology* **100**, 99–122.

GOODFELLOW, M. & MINNIKIN, D. E. (1978). Numerical and chemical methods in the classification of *Nocardia* and related taxa. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskranheiten und Hygiene (Abteilung I)*, Supplement **6**, 43–51.

GOODFELLOW, M. & ORCHARD, V. A. (1974). Antibiotic sensitivity of some nocardioform bacteria and its value as a criterion for taxonomy. *Journal of General Microbiology* **83**, 375–387.

GOODFELLOW, M., LIND, A., MORDARSKA, H., PATTYN, S. & TSUKAMURA, M. (1974). A cooperative numerical analysis of cultures considered to belong to the '*rhodochrous*' taxon. *Journal of General Microbiology* **85**, 291–302.

GOODFELLOW, M., ORLEAN, P. A. B., COLLINS, M. D. & ALSHAMAONY, L. (1978). Chemical and numerical taxonomy of strains received as *Gordona aurantiaca*. *Journal of General Microbiology* **109**, 57–68.

GORDON, R. E. (1966). Some criteria for the recognition of *Nocardia madurae* (Vincent) Blanchard. *Journal of General Microbiology* **45**, 355–364.

GORDON, R. E. (1967). The taxonomy of soil bacteria. In *The Ecology of Soil Bacteria*, pp. 293–321. Edited by T. R. G. Gray & D. Parkinson. Liverpool: Liverpool University Press.

GORDON, R. E. & HORAN, A. C. (1968). *Nocardia dassonvillei*, a replica of *Streptomyces griseus*. *Journal of General Microbiology* **50**, 235–240.

GORDON, R. E. & MIHM, J. M. (1962). Identification of *Nocardia caviae* (Erikson) nov. comb. *Annals of the New York Academy of Sciences* **98**, 628–636.

GREGORY, P. H. & LACEY, M. E. (1963). Mycological examination of dust from mouldy hay associated with farmer's lung disease. *Journal of General Microbiology* **30**, 75–88.

HOLMBERG, K. & HALLANDER, H. O. (1973). Numerical taxonomy and laboratory identification of *Bacterionema matruchotii*, *Rothia dentocariosa*, *Actinomyces naeslundii*, *Actinomyces viscosus*, and some related bacteria. *Journal of General Microbiology* **76**, 43–63.

JEFFRIES, C. D., HOLTMAN, D. F. & GUSE, D. G. (1957). Rapid method for determining the activity of micro-organisms on nucleic acids. *Journal of Bacteriology* **73**, 590–591.

JONES, D. (1975). A numerical taxonomic study of coryneform and related bacteria. *Journal of General Microbiology* **87**, 52–96.

KURUP, P. V. & SCHMITT, J. A. (1973). Numerical taxonomy of *Nocardia*. *Canadian Journal of Microbiology* **19**, 1035–1048.

LACEY, J. (1971). The microbiology of moist barley storage in unsealed silos. *Annals of Applied Biology* **69**, 187–212.

LACEY, J. (1974). Moulding of sugar-cane bagasse and its prevention. *Annals of Applied Biology* **76**, 63–67.

LACEY, J. (1978). Ecology of actinomycetes in fodders and related substrates. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I)*, Supplement **6**, 161–170.

LACEY, J. & GOODFELLOW, M. (1975). A novel actinomycete from sugar-cane bagasse: *Saccharopolyspora hirsuta* gen. et sp. nov. *Journal of General Microbiology* **88**, 75–85.

LACEY, J., GOODFELLOW, M. & ALDERSON, G. (1978). The genus *Actinomadura* Lechevalier and Lechevalier. *Zentralblatt für Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene (Abteilung I)*, Supplement **6**, 107–117.

LAVROVA, N. V., PREOBRAZHENSKAYA, T. P. & SVESHNIKOVA, M. A. (1972). Isolation of actinomycetes on selective media with rubomycin. *Antibiotiki* **17**, 965–970.

LECHEVALIER, M. P. & GERBER, N. N. (1970). The identity of madurose with 3-*O*-methyl-D-galactose. *Carbohydrate Research* **13**, 451–454.

LECHEVALIER, H. A. & LECHEVALIER, M. P. (1970). A critical evaluation of the genera of aerobic actinomycetes. In *The Actinomycetales*, pp. 393–405. Edited by H. Prauser. Jena: Gustav Fischer Verlag.

LECHEVALIER, H. A., LECHEVALIER, M. P. & GERBER, N. N. (1971). Chemical composition as a criterion in the classification of actinomycetes. *Advances in Applied Microbiology* **14**, 47–72.

LECHEVALIER, M. P., DE BIEVRE, C. & LECHEVALIER, H. (1977). Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochemical Systematics and Ecology* **5**, 249–260.

MCCLUNG, N. M. (1974). Nocardiaceae Castellani and Chalmers 1919. In *Bergey's Manual of Determinative Bacteriology*, 8th edn, pp. 726–746. Edited by R. E. Buchanan & N. E. Gibbons. Baltimore: Williams & Wilkins.

MEYER, J. (1976). *Nocardiopsis*, a new genus of the order Actinomycetales. *International Journal of Systematic Bacteriology* **26**, 487–493.

MINNIKIN, D. E., ALSHAMAONY, L. & GOODFELLOW, M. (1975). Differentiation of *Mycobacterium, Nocardia* and related taxa by thin-layer chromatographic analysis of whole-organism methanolysates. *Journal of General Microbiology* **88**, 200–204.

MINNIKIN, D. E., PIROUZ, T. & GOODFELLOW, M. (1977). Polar lipid composition in the classification of some *Actinomadura* species. *International Journal of Systematic Bacteriology* **27**, 118–121.

MORDARSKI, M., GOODFELLOW, M., SZYBA, K., PULVERER, G. & TKACZ, A. (1977). Classification of the '*rhodochrous*' complex and allied taxa based upon deoxyribonucleic acid reassociation. *International Journal of Systematic Bacteriology* **27**, 31–37.

NONOMURA, H. & OHARA, Y. (1971). Distribution of actinomycetes in soil. XI. Some new species of the genus *Actinomadura* Lechevalier *et al. Journal of Fermentation Technology* **49**, 904–912.

PRAUSER, H. (1976). Host–phage relationships in nocardioform organisms. In *The Biology of the Nocardiae*, pp. 266–284. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.

PRIDHAM, T. G. & LYONS, A. J., JR. (1969). Progress in classification of the taxonomic and nomenclatural status of some problem actinomycetes. *Developments in Industrial Microbiology* **10**, 183–221.

SCHAAL, K. P. (1977). *Nocardia, Actinomadura* and *Streptomyces*. In *CRC Handbook Series in Clinical Laboratory Sciences, Section E, Clinical Microbiology*, vol. 1, pp. 131–144. Edited by A. von Graevenitz. Ohio: CRC Press.

SCHNEIDAU, J. D., JR & SHAFFER, M. F. (1957). Studies on *Nocardia* and other Actinomycetales. *Annual Review of Tuberculosis* **76**, 770–788.

SNEATH, P. H. A. (1957). The application of computers to taxonomy. *Journal of General Microbiology* **17**, 201–226.

SNEATH, P. H. A. (1968). Vigour and pattern in taxonomy. *Journal of General Microbiology* **54**, 1–11.

SNEATH, P. H. A. & JOHNSON, R. (1972). The influence on numerical taxonomic similarities of errors in microbiological tests. *Journal of General Microbiology* **72**, 377–392.

SNEATH, P. H. A. & SOKAL, R. R. (1974). *Numerical Taxonomy. The Principles and Practice of Numerical Classification*. San Francisco: W. H. Freeman.

SNEATH, P. H. A. & STEVENS, M. (1967). A divided petri dish for use with multipoint inoculators. *Journal of Applied Bacteriology* **30**, 495–497.

SOKAL, R. R. & MICHENER, C. D. (1958). A statistical method for evaluating systematic relationships. *Kansas University Science Bulletin* **38**, 1409–1438.

TSUKAMURA, M. (1969). Numerical taxonomy of the genus *Nocardia*. *Journal of General Microbiology* **56**, 265–287.

WISHART, D. (1968). *A Fortran II Program for Numerical Taxonomy*. St Andrews: University of St Andrews.

WILLIAMS, S. T., SHARPLES, G. P., SERRANO, J. A., SERRANO, A. A. & LACEY, J. (1976). The micromorphology and fine structure of nocardioform organisms. In *The Biology of the Nocardiae*, pp. 102–140. Edited by M. Goodfellow, G. H. Brownell & J. A. Serrano. London: Academic Press.