Numerical Classification of *Mycobacterium farcinogenes*, *Mycobacterium senegalense* and Related Taxa

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Sixteen strains designated *Mycobacterium farcinogenes*, fifteen *Mycobacterium senegalense*, and ten *Nocardia farcinica* were, together with strains of *Mycobacterium* and *Nocardia*, subjected to numerical phenetic analyses using 96 unit characters. The data were examined using the simple matching (S_{SM}), Jaccard (S_J) and pattern (D_P) coefficients and clustering achieved using the unweighted average linkage algorithm. Cluster composition was not markedly affected by the coefficient used or by test error, estimated at 2.5%. The *N. farcinica* strains formed a distinct and homogeneous cluster in an aggregate taxon corresponding to the genus *Nocardia*. The *M. farcinogenes* and *M. senegalense* strains were recovered in well-defined and homogeneous phenons within the genus *Mycobacterium*. *Mycobacterium senegalense* consistently showed a high overall similarity with clusters equated with *M. chelonae* and *M. fortuitum*, while the *M. farcinogenes* cluster was not closely associated with any of the mycobacterial clusters. The results are discussed in the light of other developments in the taxonomy of the bovine farcy bacteria.

INTRODUCTION

Mycobacterium farcinogenes was proposed by Chamoiseau (1973) who recognized two subspecies, *tchadense* and *senegalense*, which encompassed strains able to cause farcy, a serious disease of African bovines (Chamoiseau, 1969, 1972). The subspecies were subsequently raised to species status as *Mycobacterium farcinogenes* and *Mycobacterium senegalense*, respectively (Chamoiseau, 1979), and were distinguished on the basis of DNA homology, growth rate, biochemical activity and histopathological behaviour. In the absence of comparative studies little is known, however, of the relationship of *M. farcinogenes* and *M. senegalense* to established species of *Mycobacterium*, or to *Nocardia farcinica* Trevisan which has been confused with the bovine farcy strains (Magnusson & Mariat, 1968; Ridell, 1975; Magnusson, 1976; Orchard & Goodfellow, 1980).

Chemical and serological analyses show that *N. farcinica* can be clearly distinguished from both *M. farcinogenes* and *M. senegalense* (Ridell, 1981; Ridell *et al.*, 1982). Thus, strains of the former contain a single mycolic acid methyl ester and non-hydroxylated fatty acid methyl esters characteristic of true nocardiae (Minnikin & Goodfellow, 1980) and have more precipitinogens in common with nocardiae, as opposed to mycobacteria. However, nocardiae and mycobacteria have many physiological and biochemical properties in common and simple diagnostic tests are needed to distinguish *N. farcinica* from the two mycobacterial taxa. Lipid and serological data also suggest that *M. farcinogenes* and *M. senegalense* are closely related to one another and to *M. fortuitum*, *M. phlei* and *M. smegmatis*. On the basis of mycolic acid analysis, *M. farcinogenes* and *M. senegalense* cannot be separated as each contains α -mycolates and characteristic polar mycolates previously only found in *M. fortuitum* and *M. smegmatis*. The serological data reinforce these affinities and also suggest that *M. farcinogenes* and *M. senegalense* might be classified in a

Abbreviation: L-J, Löwenstein-Jensen.

single species. Ridell and her colleagues concluded, however, that detailed studies using other modern taxonomic methods were required to unravel the relationship between the two bovine farcy taxa.

Numerical phenetic surveys, with their emphasis on many independent characters, have established a framework for the intrageneric classification of the genera *Mycobacterium* (Goodfellow & Wayne, 1982) and *Nocardia* (Orchard & Goodfellow, 1980; Goodfellow & Minnikin, 1981*a*). Numerical taxonomy also gives results that are valuable for identification, and a computer probability matrix exists for the identification of unknown slow growing mycobacteria (Wayne *et al.*, 1980). In the present study, good representative strains of *M. farcinogenes*, *M. senegalense* and *N. farcinica*, and marker strains of related taxa, were compared using numerical taxonomic techniques.

METHODS

Strains. The 82 test strains were obtained from either private or public culture collections (Table 1). Where possible, type strains were included. More detailed information on the strains can be found elsewhere (Ridell, 1975; Orchard & Goodfellow, 1980; Ridell *et al.*, 1982). All of the cultures were maintained on Löwenstein-Jensen (L-J) slopes at room temperature.

Media. The L-J media, Rogosa medium, Sabouraud agar, blood agar and gelatin were all provided by Dr E. Falsen, Section for Microbiological Media, Institute of Medical Microbiology, Göteborg, Sweden. The glucose nutrient agar was based on material from Oxoid.

Collection of data. Each strain (*t*) was examined for 108 unit characters (*n*; Table 2). Unless otherwise stated, the strains were incubated at 37 °C and examined after 7, 14, 21, or in the case of poorly growing cultures, 28 d. Data for computation were taken from final test readings.

Colony morphology and pigmentation. Colonial properties were observed on glucose nutrient agar plates.

Biomedical and degradative tests. Cultures were examined for 3 d arylsulphatase (Wayne, 1961), catalase 68 °C (Kubica & Pool, 1960; David, 1976), β -lactamase (Backelin *et al.*, 1973), nitrophenoloxidase (Bönicke & Juhasz, 1965) and nitrate reductase (Virtanen, 1960; David, 1976). Adenine (0.5%, w/v), elastin (0.1%, w/v), guanine (0.2%, w/v), hypoxanthine (0.5%, w/v), testosterone (0.1%, w/v), tyrosine (0.5%, w/v) and xanthine (0.4%, w/v) were examined in Gordon's (1967) basal medium. In these tests, the disappearance of the insoluble compounds from underneath and around areas of growth was scored as a positive result. Starch (1%, w/v) decomposition was detected after Gordon & Mihm (1957). The haemolysis test was performed using broth agar enriched with defibrinated horse blood (10%, v/v).

Inhibition tests. Resistance to lysozyme and rifampin was performed after Gordon & Barnett (1977). The ability of the strains to grow in the presence of various concentrations of the remaining antimicrobial and chemical agents was examined using L-J and glucose nutrient agar plates, respectively.

Tolerance tests. Cultures were examined for their ability to grow at 12 °C and 50 °C on glucose and nutrient agar and at 45 °C on L-J medium. Survival at 60 °C was determined by inoculating glucose broths, incubating at 37 °C until good growth was obtained, then heating the broth suspensions in a water bath at 60 °C for 4 h before plating out a loopful of the heated suspensions on to L-J medium and incubating at 37 °C. Tolerance to pH was examined in glucose nutrient broth supplemented with horse serum (13%, v/v) and buffered with phosphate buffers.

Growth tests. The organisms were examined for their ability to grow on Sabouraud agar, Rogosa medium and in gelatin broth. Visible growth after 24 h and 5 d and the number of colonies on plates were determined by inoculating glucose nutrient agar with 0.1 ml of a suspension containing a loopful (4 mm diam. loop) of bacteria suspended in 2 ml phosphate buffer.

Coding of data. Most of the characters existed in one of two mutually exclusive states and were scored plus (+) or minus (-). Qualitative multistate characters, such as pigmentation and some of the colony characters, were scored plus for the character state exhibited and minus for the alternatives. Quantitative multistate characters, mainly derived from the biochemical and inhibition tests, were scored using the additive method of Sneath & Sokal (1973). Resistance to the antimicrobial and chemical agents was scored as +. Twelve unit characters were deleted from the data matrix as all of the strains gave identical (+ + or - -) results. The final *n* $\times$ *t* table, therefore, contained data for 82 strains and 96 unit characters. The binary data were stored on standard IBM punch cards.

Computation. Data were analysed using the Clustan 1A program (Wishart, 1978) on an IBM 370/180 computer using the simple matching coefficient (S_{SM} ; Sokal & Michener, 1958), which includes both positive and negative matches, the Jaccard coefficient (S_J ; Sneath, 1957) which counts positive matches only, and the pattern coefficient (D_P ; Sneath, 1968), which seeks to allow for differences in growth rates, incubation periods and similar factors which might contribute to overall dissimilarity between strains. In all cases, data were sorted using the unweighted average linkage (UPGMA) algorithm (Sneath & Sokal, 1973) and presented as abbreviated dendrograms (Figs 1-3).

Table 1. Designation and source of strains assigned to clusters in the S_{SM} /UPGMA analysis

Strains assigned to cluster 1 (<i>Mycobacterium marinum</i>)	
*GA411	<i>Mycobacterium marinum</i> , ATCC 927
GA012	<i>M. marinum</i> , L. Zettergren, University of Göteborg, Göteborg, Sweden
Strains assigned to cluster 2 (<i>Mycobacterium avium</i>)	
GA009	<i>Mycobacterium avium</i> , G. Penso, Istituto Superiore di Sanità, Rome, Italy, Ceppo Faisan IV
GA655	<i>M. avium</i> , ATCC 19421
Strains assigned to cluster 3 (<i>Mycobacterium scrofulaceum</i>)	
GA027	<i>Mycobacterium scrofulaceum</i> , G. Penso, Ceppo chauviré
GA631	<i>M. scrofulaceum</i> , ATCC 25214
Strains assigned to cluster 4 (<i>Mycobacterium kansasii</i>)	
GA120	<i>Mycobacterium kansasii</i> , E. H. Runyon, Salt Lake City, Utah, U.S.A., P16
GA670	<i>M. kansasii</i> , ATCC 25221
Strains assigned to cluster 5 (<i>Mycobacterium vaccae</i>)	
*GA681	<i>Mycobacterium vaccae</i> , ATCC 15483
GA333,	<i>M. vaccae</i> , R. N. Bönicke, Forschungsinstitut Borstel, F.R.G.,
GA335	SN901, SN967
Strains assigned to cluster 6 (<i>Mycobacterium gastri</i>)	
*GA363	<i>Mycobacterium gastri</i> , ATCC 15754
GA447	<i>M. gastri</i> , L. G. Wayne, Veterans Administration Hospital, Long Beach, California, U.S.A., W465
Strains assigned to cluster 7 (<i>Mycobacterium farcinogenes</i>)	
GA922	<i>Mycobacterium farcinogenes</i> , M. P. Lechevalier, Rutgers University, New Brunswick, N.J., U.S.A., IP740. M. Goodfellow, M261
GA921	<i>M. farcinogenes</i> , M. P. Lechevalier, IP736. M. Goodfellow, M260
GA950	<i>M. farcinogenes</i> , E. H. Runyon R428; IP743. M. Goodfellow, M276
*GA923	<i>M. farcinogenes</i> , NCTC 10955. M. Goodfellow, M262
GA948	<i>M. farcinogenes</i> , E. H. Runyon, R415; R. E. Gordon, 1226. M. Goodfellow, M271
GB047	<i>M. farcinogenes</i> , M. P. Lechevalier, 1234. M. Goodfellow, N716
GA949	<i>M. farcinogenes</i> , E. H. Runyon, R425; IP738. M. Goodfellow, M272
GA951	<i>M. farcinogenes</i> , E. H. Runyon, R429; IP744. M. Goodfellow, M277
GA959	<i>M. farcinogenes</i> , E. H. Runyon, R422; IP735. M. Goodfellow, M269
GB052	<i>M. farcinogenes</i> , M. P. Lechevalier, 1244. M. Goodfellow, N720
GA967	<i>M. farcinogenes</i> , E. H. Runyon, R414; IP739. M. Goodfellow, M273
GB042	<i>M. farcinogenes</i> , R. E. Gordon, Rutgers University, New Brunswick, N.J., U.S.A., 1242. M. Goodfellow, N711
GB040	<i>M. farcinogenes</i> , R. E. Gordon, 1223. M. Goodfellow, N709
GA968	<i>M. farcinogenes</i> , E. H. Runyon, R427; IP742. M. Goodfellow, N275
GB046	<i>M. farcinogenes</i> , M. P. Lechevalier, 1224. M. Goodfellow, N715
Strains assigned to cluster 8 (<i>Mycobacterium senegalense</i>)	
GA969	<i>Mycobacterium farcinogenes</i> , E. H. Runyon, R147. M. Goodfellow, M280
GB045	<i>Mycobacterium senegalense</i> , R. E. Gordon, 1363. M. Goodfellow, N714
GA932	<i>M. senegalense</i> , E. H. Runyon, R456. M. Goodfellow, M283
GA882	<i>M. senegalense</i> , M. Magnusson, Statens Seruminstitut, Copenhagen, Denmark, 875. M. Goodfellow, N727
GA931	<i>M. senegalense</i> , E. H. Runyon, R455. M. Goodfellow, M282
GA883	<i>M. senegalense</i> , M. Magnusson, 876. M. Goodfellow, N728
GA939	<i>M. senegalense</i> , E. H. Runyon, R408; R. E. Gordon, 1361, IP397. M. Goodfellow, M265
GA940	<i>M. senegalense</i> , E. H. Runyon, R409; R. E. Gordon, 1362; IP443C
*GA924	<i>M. senegalense</i> , NCTC 10956. M. Goodfellow, M263
GA884	<i>M. senegalense</i> , E. H. Runyon, 410; R. E. Gordon, 1364. M. Goodfellow, N729
GB053	<i>M. senegalense</i> , M. P. Lechevalier, 931. M. Goodfellow, N721
GA935	<i>M. senegalense</i> , E. H. Runyon, R148. M. Goodfellow, M279
GA920	<i>M. senegalense</i> , NCTC 4524. M. Goodfellow, M259
GA786	<i>M. senegalense</i> , I. Juhlin, Malmö allmänna sjukhus, Malmö, Sweden 396/193. M. Goodfellow, N696
GA816	<i>M. senegalense</i> , R. N. Bönicke, SN5701. M. Goodfellow, N723
GB043	<i>M. senegalense</i> , R. E. Gordon, 1360. M. Goodfellow, N712

Table 1. (continued)

Strains assigned to cluster 9 (*Mycobacterium chelonae*)

- *GA717 *Mycobacterium chelonae*, NCTC 946
 GA716 *M. chelonae*, ATCC 19977

Strains assigned to cluster 10 (*Mycobacterium fortuitum*)

- GA728 *Mycobacterium fortuitum*, NCTC 2291; ATCC 9821
 GA730 *M. fortuitum*, NCTC 10395; ATCC 6842
 *GB178 *M. fortuitum*, NCTC 10394; ATCC 6841
 GA023 *M. fortuitum*, G. Penso, 456

Strains assigned to cluster 11 (*Mycobacterium phlei*)

- *GB191 *Mycobacterium phlei*, NCTC 8151; ATCC 11758
 GA732 *M. phlei*, NCTC 8156
 GA733 *M. phlei*, NCTC 10260

Strains assigned to cluster 12 (*Mycobacterium smegmatis*)

- GB734 *Mycobacterium smegmatis*, NCTC 3821
 *GB735 *M. smegmatis*, NCTC 8159; ATCC 19420
 GB736 *M. smegmatis*, NCTC 10265; ATCC 14468

Strains assigned to cluster 13 (*Nocardia farcinica*)

- *GA819 *Nocardia farcinica*, R. N. Bönicke, EB1957; ATCC 3318. M. Goodfellow, N699
 *GA826 *N. farcinica*, M. Magnusson, 752; ATCC 3318. M. Goodfellow, N700
 *GA919 *N. farcinica*, M. P. Lechevalier, ATCC 3318. M. Goodfellow, M258
 GA879 *N. farcinica*, M. Magnusson, 654; ATCC 6864. M. Goodfellow, N704
 GA886 *N. farcinica*, M. Magnusson, 952
 GA827 *N. farcinica*, M. Magnusson, 753; ATCC 3399. M. Goodfellow, N701
 GA880 *N. farcinica*, M. Magnusson, 884. M. Goodfellow, N705
 GA885 *N. farcinica*, M. Magnusson, 878. M. Goodfellow, N707
 GB293 *N. farcinica*, M. Magnusson, 845
 GA988 *Nocardia asteroides*, S. G. Bradley, Virginia Commonwealth University, Richmond, Va, U.S.A.,
 VAC 426. M. Goodfellow, N668
 GB002 *N. asteroides*, M. Goodfellow, N906
 GA991 *N. farcinica*, S. G. Bradley, VAC 324. M. Goodfellow, N670

Strains assigned to cluster 14 (*Nocardia asteroides*)

Subcluster 14A

- *GA873 *Nocardia otitidis-caviarum*, ATCC 14629. M. Goodfellow, N36
 GA852 *N. otitidis-caviarum*, R. E. Gordon, 737
 GA869 *Nocardia asteroides*, R. E. Gordon, NCTC 659

Subcluster 14B

- GA987 *N. asteroides*, M. Goodfellow, N861
 GA990 *N. asteroides*, K. P. Schaal, Hygiene-Institute, University of Cologne, F.R.G., N23.
 M. Goodfellow, N692

Subcluster 14C

- GA984 *N. asteroides*, K. P. Schaal, N2. M. Goodfellow, N688
 GA985 *N. asteroides*, K. P. Schaal, N3. M. Goodfellow, N689
 GA999 *N. asteroides*, K. P. Schaal, N10. M. Goodfellow, N904
 GA986 *N. asteroides*, NCTC 8595; ATCC 14759. M. Goodfellow, N13

Subcluster 14D

- *GA875 *N. asteroides*, ATCC 19247. M. Goodfellow, N317
 GA979 *N. asteroides*, M. Hutchinson, Water Research Centre, Medmenham, Bucks., U.K.
 M. Goodfellow, E9

Strains assigned to cluster 15 (*Nocardia brasiliensis*)

- *GA976 *Nocardia brasiliensis*, ATCC 19296. M. Goodfellow, N318
 GA982 *N. brasiliensis*, NCTC 10300; ATCC 19295. M. Goodfellow, N14

Single member cluster

- GB003 *Nocardia asteroides*, K. P. Schaal, N60. M. Goodfellow, N907

* Type strains. Sources: ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; IP, Institut Pasteur, Paris, France; NCTC, National Collection of Type Cultures, London, U.K.

Fig. 1. A simplified dendrogram showing relationships between clusters and subclusters based on the S_{SM} coefficient and unweighted average linkage algorithm.

RESULTS

Test error and final data matrix

The inclusion of three strains of *N. farcinica* ATCC 3318 in the study (Table 1) made it possible to get an approximate estimate of the experimental test error. The triplicate cultures united at the 95% similarity (S) level indicating that the average probability (P) of an erroneous test result was around 2.5%. Twelve tests were deleted from the data matrix as they had no separation value. None of the strains degraded adenine, blood, elastin, guanine or starch, nor were they able to grow at pH 3.3, pH 11.3, or on Rogosa medium. In contrast, all of the cultures grew in the presence of crystal violet (0.0001%, w/v), phenol (0.001, 0.01%, w/v) and sodium azide (0.001%, w/v).

Clustering of strains using the S_{SM} coefficient with the UPGMA algorithm

Eighty-one of the eighty-two strains were recovered in fifteen clusters defined at the 82% S -level (Table 1; Fig. 1). At the 66% S -level the *Mycobacterium* and *Nocardia* strains separated into two aggregate clusters. The nocardiae fell into three clusters, the largest of which was equated with *N. farcinica*. Thus, cluster 13 contained all 10 strains of *N. farcinica* and *N. asteroides* GA988 and GB002. This cluster was clearly separated from all of the remaining phenotypes including those equated with *N. asteroides* and *N. brasiliensis*. The *N. asteroides* cluster was divided into four subclusters one of which, 14A, contained *N. otitidis-caviarum* GA852 and GA873 and *N. asteroides* GA896. The final strain received as *N. asteroides*, GB003, formed a single member cluster which shared its highest affinities with strains assigned to cluster 14.

Fig. 2. A simplified dendrogram showing the relationships between clusters and subclusters based on the D_p coefficient and unweighted average linkage algorithm.

The 56 mycobacteria fell into 12 distinct clusters (Fig. 1). The largest, cluster 7, accommodated 15 of the 16 strains received as *M. farcinogenes*. These strains fused at the 85% S -level to form a distinct and homogeneous taxon which shared its highest similarities with phena containing slow growing marker strains of *M. avium*, *M. gastri*, *M. kansasii*, *M. marinum* and *M. scrofulaceum*. Cluster 5, which contained the three *M. vaccae* markers, also clustered with the phena encompassing the slow growing strains. The remaining fast growing mycobacteria were recovered in clusters assigned to two broad groups. The first, formed at the 76% S -level, included clusters 8, 9 and 10, each of which was defined at the 85% S -level, and were equated with *M. senegalense*, *M. chelonae* and *M. fortuitum*, respectively. Cluster 8 contained all 15 strains of *M. senegalense* and the remaining strain (GA969) received as *M. farcinogenes*. The marker strains of *M. phlei* and *M. smegmatis* formed clusters 11 and 12 which united at the 80% S -level to give the second group.

Clustering of strains using the D_p and S_j coefficient with the UPGMA algorithm

The composition of the clusters obtained in the D_p /UPGMA analysis differed only marginally from that obtained in the corresponding S_{SM} analysis. The composition of both the clusters and subclusters recovered in the S_j analysis was identical to that found in the S_{SM} /UPGMA analysis. The arrangement of clusters was, however, influenced by the resemblance coefficient used (Figs 1 and 3). In the D_p analysis, the *Mycobacterium* and *Nocardia* strains were again separated into aggregate phena but the homogeneous and distinct *M. farcinogenes* phenon shared a marginally higher affinity with the *M. chelonae*/*M. fortuitum*/*M. senegalense* group than with the taxon composed of the *M. avium*, *M. kansasii*, *M. marinum* and *M. scrofulaceum* clusters (Fig. 2). Further, the *M. vaccae* cluster was no longer classified with

Fig. 3. A simplified dendrogram showing the relationships between clusters and subclusters based on the S_j coefficient and unweighted average linkage algorithm.

these slow growing taxa, but was associated with the *M. phlei*/*M. smegmatis* group. In the S_j analysis the *Nocardia* strains formed a recognizable aggregate phenon which split the *Mycobacterium* clusters into two parts (Fig. 3). The *M. farcinogenes* cluster was again marginally closer to the *M. chelonae*/*M. fortuitum*/*M. senegalense* group than to the phenon composed of the *M. avium*, *M. kansasii*, *M. marinum* and *M. scrofulaceum* clusters. The *M. vaccae* cluster was now loosely associated with the *M. chelonae* group, while *M. gastri* was recovered as a distinct cluster.

Of the eighty-two test strains, only five were assigned to different clusters in the D_p analysis as compared with the S_{SM} /UPGMA classification. The *M. fortuitum* cluster was, however, recovered as two subclusters in the D_p /UPGMA study. Subcluster 10A contained *M. fortuitum* GA023, *M. senegalense* GA816 and GB043 and subcluster 10B the three remaining *M. fortuitum* strains and *M. gastri* GA363. The second *M. gastri* strain, GA447, joined the *M. senegalense* strains in cluster 8. The final strain, *N. asteroides* GA003, previously a single member cluster, fell into subcluster 14B which also contained *N. asteroides* GA986 from subcluster 14C. *Nocardia asteroides* GA869, classified with the *N. otitidis-caviarum* strains of subcluster 14A in the earlier analysis, now joined the type strain of *N. asteroides* (GA875) to give subcluster 14D. *Nocardia asteroides* GA979, previously with subcluster 14D, fell into subcluster 14C.

Characterization of *Mycobacterium farcinogenes*, *Mycobacterium senegalense* and related taxa

The characteristics of *N. farcinica* and the *Mycobacterium* species defined in the S_{SM} /UPGMA analysis are shown in Table 2. Characters potentially useful for differentiating *M. farcinogenes* and *M. senegalense* from one another and from some related taxa are shown in Table 3.

Table 2. *Comparison of the characteristics of Mycobacterium farcinogenes, Mycobacterium senegalense and some related taxa*

	<i>M. avium</i>	<i>M. gastri</i>	<i>M. kansasii</i>	<i>M. marinum</i>	<i>M. scrofulaceum</i>	<i>M. farcinogenes</i>	<i>M. senegalense</i>	<i>M. chelonae</i>	<i>M. fortuitum</i>	<i>M. phlei</i>	<i>M. smegmatis</i>	<i>M. vaccae</i>	<i>N. farcinica</i>
Cluster no. . . .	2	6	4	1	3	7	8	9	10	11	12	5	13
No. of strains . . .	2	2	2	2	2	15	16	2	4	3	3	3	12
Character*													
Colonial characters:													
Colonies white/grey	2	2	2	0	0	15	16	2	3	0	0	0	0
Colonies yellow	0	0	0	2	2	0	0	0	1	3	3	3	10
Colonies orange/red	0	0	0	0	0	0	0	0	0	0	0	0	2
Colonies ≤ 2 mm diam.	2	0	2	1	1	8	1	0	0	0	0	0	0
Colonies ≤ 10 mm diam.	0	0	0	0	0	0	0	0	0	1	1	0	1
Aerial hyphae sparse	0	0	0	0	0	10	9	0	0	0	0	0	8
Aerial hyphae moderate	0	0	0	0	0	3	0	0	0	0	0	0	6
Aerial hyphae abundant	0	0	0	0	0	0	0	0	0	0	0	0	1
Colonies glossy	1	2	1	1	2	5	11	1	4	0	3	3	8
Colonies smooth	1	0	0	1	1	0	0	1	0	0	0	3	0
Colonies folded	1	1	2	1	1	15	16	1	4	3	3	0	12
Margin elevated	0	0	0	0	0	3	0	0	0	0	0	0	4
Margin filamentous	0	0	0	0	0	0	0	0	0	0	0	0	10
Elevation concave	0	0	0	0	0	0	0	0	0	0	0	0	1
Colonies easily detached from agar	0	0	0	0	0	11	0	0	0	0	0	0	0
Growth into agar moderate	0	0	0	0	0	0	1	0	0	0	0	0	10
Growth into agar extensive	0	0	0	0	0	0	0	0	0	0	0	0	7
Colonies emulsifiable	2	2	1	1	2	0	5	2	4	2	3	3	7
Colonies highly emulsifiable	1	1	0	1	2	0	0	2	2	0	2	0	3
Colonies ropy	1	0	0	0	0	0	0	0	0	0	0	0	0
Colonies coarse	0	0	0	1	0	15	8	0	2	3	0	0	8
Colonies very coarse	0	0	0	0	0	13	1	0	0	0	0	0	0
Biochemical tests:													
Arylsulphatase	0	2	0	1	0	1	15	2	4	0	0	3	0
Catalase positive	0	0	2	2	2	0	14	0	3	2	0	3	10
Catalase strongly positive	0	0	0	0	0	0	1	0	0	0	0	1	0
β -lactamase after 20 min:													
Positive	0	1	2	0	0	10	12	2	1	2	3	0	12
Moderately positive	0	0	0	0	0	5	6	0	0	1	3	0	12
Highly positive	0	0	0	0	0	4	2	0	0	1	2	0	8
β -lactamase after 60 min:													
Positive	0	1	2	0	0	14	14	2	3	3	3	3	12
Moderately positive	0	1	0	0	0	9	13	2	2	3	3	2	12
Highly positive	0	1	0	0	0	7	8	2	1	2	3	0	12
Nitrite reductase positive	0	1	2	0	2	10	15	0	4	3	3	2	3
Nitrite reductase highly positive	0	0	2	0	0	2	13	0	4	3	2	0	0
Nitrophenoloxidase	0	0	0	0	0	0	0	0	0	0	0	0	9
Degradation tests:													
Testosterone	2	2	2	0	2	13	16	0	4	3	3	3	12
Tyrosine	0	0	0	0	0	0	0	0	0	0	0	0	1
Resistance to ($\mu\text{g ml}^{-1}$):													
Capreomycin, 10	2	0	2	2	2	0	15	2	4	0	3	3	12
20	2	0	1	0	2	0	9	2	4	0	3	2	12
40	2	0	1	0	0	0	5	2	4	0	0	0	6
160	0	0	0	0	0	0	3	2	4	0	0	0	2
320	0	0	0	0	0	0	0	2	2	0	0	0	1
Dapsone, 100	2	0	2	2	2	2	16	2	4	1	3	0	7
Ethambutol, 4	1	0	1	2	2	10	16	2	4	1	0	0	12
8	0	0	0	0	1	5	16	2	4	1	0	0	12
32	0	0	0	0	0	2	15	2	4	0	0	0	10

Table 2. (continued)

	<i>M. avium</i>	<i>M. gastri</i>	<i>M. kansasii</i>	<i>M. marinum</i>	<i>M. scrofulaceum</i>	<i>M. farcinogenes</i>	<i>M. senegalense</i>	<i>M. chelonae</i>	<i>M. fortuitum</i>	<i>M. phlei</i>	<i>M. smegmatis</i>	<i>M. vaccae</i>	<i>N. farcinica</i>
Cluster no. . . .	2	6	4	1	3	7	8	9	10	11	12	5	13
Character* No. of strains . . .	2	2	2	2	2	15	16	2	4	3	3	3	12
Isoniazid, 0.25	2	2	2	2	2	14	16	2	4	3	3	3	12
0.50	2	0	2	2	2	14	16	2	4	3	3	3	12
2.00	2	0	2	2	2	14	16	2	4	3	3	0	12
Lysozyme, 50	1	2	1	2	2	14	16	2	4	1	1	3	12
100	1	2	0	2	2	14	10	2	3	0	1	2	12
200	0	2	0	2	0	0	9	1	3	0	0	1	12
Penicillin, 66 i.u. ml ⁻¹	2	2	2	2	2	12	16	2	4	3	3	3	12
Prothionamide, 10	2	1	2	2	2	15	16	2	4	3	3	3	12
20	2	1	0	0	2	15	16	2	4	3	3	3	12
40	2	0	0	0	2	14	14	2	4	3	3	1	12
160	0	0	0	0	0	2	2	0	4	1	2	0	12
320	0	0	0	0	0	0	0	0	0	0	0	0	12
Rifampin, 20	0	0	1	0	0	9	2	1	4	0	3	0	12
Streptomycin, 8	1	0	0	2	2	12	16	2	4	0	0	0	12
16	1	0	0	0	0	5	16	2	4	0	0	0	12
64	0	0	0	0	0	0	12	2	4	0	0	0	6
Growth in presence of:													
Crystal violet (0.001%, w/v)													
Good	2	0	2	1	2	1	2	0	4	1	3	0	2
Very good	0	0	1	0	2	0	0	0	4	0	2	0	0
Phenol (0.1%, w/v)													
Good	0	0	0	0	0	0	0	0	4	3	3	0	12
Very good	0	0	0	0	0	0	0	0	0	3	3	0	12
Sodium azide (0.01%, w/v)													
Good	0	0	1	0	0	0	4	0	4	3	3	2	12
Very good	0	0	0	0	0	0	0	0	3	3	0	0	10
Potassium tellurite (0.04%, w/v)													
Good	0	1	0	0	0	1	16	1	4	3	3	3	12
Very good	0	1	0	0	0	0	9	0	4	2	2	2	12
Sodium chloride (3%, w/v)	0	1	1	2	2	7	16	2	4	3	3	3	12
Sodium chloride (5%, w/v)	0	1	0	0	0	0	14	1	4	3	3	2	12
Sodium chloride (7%, w/v)	0	0	0	0	0	0	1	0	3	2	2	0	11
Sodium chloride (10%, w/v)	0	0	0	0	0	0	0	0	0	0	0	0	0
Sodium chloride (L-J; 7%, w/v)	0	0	0	0	2	1	12	0	4	2	3	0	12
Growth at:													
pH 4.0	0	0	1	0	0	0	0	0	1	2	1	0	0
pH 4.4	0	0	1	1	0	0	0	1	4	2	1	1	0
pH 5.0	1	2	1	2	2	2	16	1	4	3	3	2	12
pH 9.5	0	1	1	2	0	0	2	0	4	3	3	3	12
pH 10.2	0	1	1	0	0	0	0	0	3	2	3	1	3
pH 10.8	0	0	1	0	0	0	0	0	1	2	1	1	0
12 °C, Good	0	0	0	0	1	1	4	0	1	0	3	3	4
Very good	0	0	0	0	0	1	0	0	0	0	1	2	0
50 °C, Good	1	0	2	0	1	1	3	0	2	3	3	0	5
Very good	1	0	0	0	0	1	0	0	0	3	3	0	5
45 °C, (L-J)	2	0	0	0	1	0	0	0	0	2	3	0	12
Growth on:													
Sabouraud agar, Good	2	2	2	1	2	2	16	1	4	3	3	1	12
Very good	2	2	0	0	1	1	16	1	4	2	3	1	12
Growth after:													
24 h	0	0	0	0	0	0	2	0	4	3	3	2	10
5 d	1	0	1	0	0	0	16	0	4	3	3	3	12

Table 2. (continued)

	<i>M. avium</i>	<i>M. gastri</i>	<i>M. kansasii</i>	<i>M. marinum</i>	<i>M. scrofulaceum</i>	<i>M. farcinogenes</i>	<i>M. senegalense</i>	<i>M. chelonae</i>	<i>M. fortuitum</i>	<i>M. phlei</i>	<i>M. smegmatis</i>	<i>M. vaccae</i>	<i>N. farcinica</i>
Cluster no. . . .	2	6	4	1	3	7	8	9	10	11	12	5	13
Character* No. of strains . . .	2	2	2	2	2	15	16	2	4	3	3	3	12
No. of colonies (≤ 20) after 5 d	0	0	0	0	0	6	0	0	0	0	0	0	0
Growth in:													
Gelatin broth	0	0	0	0	0	11	16	2	4	3	3	3	12
Survival at 60 °C, 4 h	2	0	0	0	2	0	0	0	2	3	0	0	11

* None of the strains degraded adenine, blood, elastin, guanine, hypoxanthine, starch or xanthine, produced diffusible exopigments or brown colonies, grew at pH 3.3 or 11.3, grew on Rogosa medium, but all grew in the presence of crystal violet (0.0001%, w/v), phenol (0.01, 0.001%, w/v) or sodium azide (0.001%, w/v).

Table 3. Characters with the greatest resolving power for differentiating *Mycobacterium farcinogenes*, *Mycobacterium senegalense* and some related taxa

Key: +, more than 85% of strains positive; -, more than 85% of strains negative; d, strains 16 to 84% positive.

Character	<i>M. chelonae</i>	<i>M. fortuitum</i>	<i>M. farcinogenes</i>	<i>M. senegalense</i>	<i>N. farcinica</i>
Colonies white/grey	+	d	+	+	-
Arylsulphatase	+	+	-	+	-
Catalase positive	-	d	-	+	+
Testosterone	-	+	+	+	+
Sensitivity to ($\mu\text{g ml}^{-1}$)					
Capreomycin, 10	+	+	-	+	+
Dapsone, 100	+	+	-	+	d
Ethambutol, 32	+	+	-	+	+
Prothionamide, 160	-	+	-	-	+
320	-	+	-	+	+
Sodium chloride (5%, w/v)	-	+	-	+	+
Potassium tellurite (0.04%, w/v)	d	+	-	+	+
Growth at pH 5.0	d	+	-	+	+
Growth on Sabouraud agar	d	+	-	+	+
Growth after 5 d	-	+	-	+	+

DISCUSSION

The numerical phenetic classification obtained was by and large the same irrespective of the resemblance coefficient used (D_P , S_J , S_{SM}) and was only marginally affected by the test error of around 2.5%. Agreement between classifications based on different numerical techniques is a sign of good taxonomy (Austin & Colwell, 1977; Goodfellow *et al.*, 1978) and means that the numerical data can be interpreted with confidence.

In all three analyses, the *Nocardia* strains, including *N. farcinica*, formed an aggregate phenon distinct from the mycobacteria. The recovery of *N. farcinica* among the nocardiae and *M. farcinogenes* and *M. senegalense* with the mycobacteria is in agreement with previous chemical and serological analyses (Tsukamura, 1969; Ridell, 1975; Orchard & Goodfellow, 1980; Ridell *et al.*, 1982). It was also encouraging to find that these taxa can be readily distinguished by a number of biochemical and physiological tests.

The relationships between the *Mycobacterium* clusters were to some extent affected by the coefficients employed, though in all cases relatively high overall similarities were found between *M. chelonae*, *M. fortuitum* and *M. senegalense*, and between *M. phlei* and *M. smegmatis* as well as between *M. avium*, *M. kansasii*, *M. marinum* and *M. scrofulaceum*. The *M. farcinogenes* phenon was not closely related to any of these aggregate groups. It was marginally associated with the taxa containing slow growing mycobacteria in the S_{SM} analysis, with *M. chelonae*, *M. fortuitum* and *M. senegalense* in the D_P analysis, but formed a distinct cluster in the S_J study. The close similarity found between *M. gastri*, *M. fortuitum* and *M. senegalense* in the D_P analysis is a reflection of the relatively small number of positive responses shown by the former. It has already been demonstrated (Goodfellow *et al.*, 1978) that when organisms which yield many positive responses are compared with less vigorous strains, the pattern difference can be artificially small.

The recovery of the *N. farcinica* strains in a homogeneous phenon distinct from *Nocardia* clusters is in excellent agreement with previous numerical phenetic classifications (Tsukamura, 1969; Schaal & Reutersberg, 1978; Orchard & Goodfellow, 1980) and with data from DNA pairing experiments (Mordarski *et al.*, 1977, 1978). The numerical data also provide further evidence of the heterogeneity of *N. asteroides* (Tsukamura, 1969; Kurup & Schmitt, 1973; Mordarski *et al.*, 1977; Schaal & Reutersberg, 1978; Orchard & Goodfellow, 1980).

The most reliable classifications are those in which relationships between taxa are supported by different kinds of independent taxonomic criteria. In mycobacterial systematics, both numerical and comparative immunodiffusion analyses have proved to be effective in the characterization and delineation of species (Lind *et al.*, 1980; Goodfellow & Wayne, 1982). The recovery of the *M. farcinogenes* and *M. senegalense* in distinct and homogeneous clusters equivalent in rank with established species of *Mycobacterium* is consistent with their recognition as distinct species (Chamoiseau, 1973; Goodfellow & Wayne, 1982). In addition, a number of biochemical, growth and inhibition tests were found to be of presumptive value for the separation of *M. farcinogenes* and *M. senegalense*. In contrast, comparative immunodiffusion studies show that *M. farcinogenes*, *M. senegalense* and *M. fortuitum* are not only very similar serologically but are difficult to separate into distinct species (Ridell, 1981; Ridell *et al.*, 1982). The affinity between these two taxa is supported by the results of mycolic acid analyses, though TLC analysis of mycobacterial mycolates does not yield data sufficiently sensitive for species definition (Minnikin & Goodfellow, 1980; Ridell *et al.*, 1982). Two dimensional TLC of whole organism methanolysates does, however, provide a reliable way of distinguishing *M. chelonae* from *M. farcinogenes*, *M. fortuitum* and *M. senegalense* and all other mycobacteria examined to date (Goodfellow & Minnikin, 1981*b*; Minnikin *et al.*, 1982).

The present study has gone some way towards clarifying the systematics of *M. farcinogenes*, *M. senegalense*, *N. farcinica* and some related taxa. Additional work is, however, required to explain the apparently conflicting results obtained in the numerical and serological studies on *M. farcinogenes* and *M. senegalense*. Representative strains of these taxa should be examined using the DNA-DNA pairing method, for especially good congruence has been found between DNA pairing and numerical phenetic data in previous studies on actinomycetes (Mordarski *et al.*, 1981).

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