A Numerical Taxonomic Study of Streptococci of Serological Group D

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(Received 20 December 1971; revised 25 April 1972)

SUMMARY

The results of 45 biochemical and physiological tests on 122 strains of streptococci of serological group D (three of which also contained the 'Q' antigen) from a variety of habitats were subjected to computer analysis. The organisms clustered into five main groups representing the species (1) *Streptococcus faecalis*, (2) *S. faecium* and *S. durans*, which we consider are best treated as one species, and (3) *S. avium*, (4) *S. bovis*, (5) *S. equinus*.

INTRODUCTION

Seven species of streptococci are now known to contain the group D antigen. These are *Streptococcus faecalis* and its varieties *S. faecalis* var. *liquefaciens* and *S. faecalis* var. *zymogenes* and *S. durans* (Lancefield, 1933); *S. faecium* (Skadhauge, 1950); *S. bovis* (Shattock, 1949); *S. equinus* (Smith & Shattock, 1962); *S. suis* (Elliott, 1966) and *S. avium*. Most strains of *S. avium* also contain the 'Q' antigen, and whether this is a 'type' or 'group' antigen is a matter of opinion (Nowlan & Deibel, 1967).

On physiological and biochemical grounds, the species *Streptococcus faecalis*, *S. faecium* and *S. durans* are classified in the enterococcus division of Sherman (1937) and *S. bovis* and *S. equinus* in the viridans division of Sherman. Nowlan & Deibel (1967) think that *S. avium* is related to the enterococcus division and Elliott (1966) has suggested that *S. suis* is taxonomically close to *S. bovis*. The status and interrelationships of all these species have been and still are controversial.

In the enterococcus division of serological group D, disagreements at the present time revolve around how distinct biotypes must be from each other to be considered separate species. This is especially so with regard to the taxonomic status of *Streptococcus faecalis*, *S. faecium* and *S. durans*. The early literature is reviewed by Deibel (1964).

In the viridans division of serological group D disagreement revolves around the status of *Streptococcus bovis* and *S. equinus* and the relationship of both to the enterococcus division. The studies of Seeley & Dain (1960) and Smith & Shattock (1962) have shown a close relationship between *S. bovis* and *S. equinus*; these authors differed only on whether the two species deserved separate status or should be regarded as biotypes of the same species. However, a numerical study by Raj & Colwell (1966) showed *S. equinus* (one strain only) to be more closely related to the pyogenic group of streptococci than to *S. bovis* or any other species in serological group D.

Medrek & Barnes (1962) suggested that *Streptococcus bovis*, as presently constituted, contains two biotypes, and this opinion receives some support from the earlier work of

Mann, Masson & Oxford (1954) and Dain, Neal & Seeley (1956), although not from the numerical study of Raj & Colwell (1966).

All early studies (reviewed by Deibel, 1964) show *Streptococcus bovis* to be biochemically distinct from the enterococcus group, but this separation is not supported by the study of Raj & Colwell (1966), where *S. bovis* is included in the enterococcus division.

Some time ago one of us (Jones, 1958) did a number of physiological and biochemical tests on 122 strains of streptococci of serological group D (three of which also contained the group Q antigen) from a variety of sources. In view of the discrepancies between intuitive and numerical studies and between different numerical studies of this group, it was considered worth while to submit the data to numerical analysis. The results are presented here.

METHODS

Strains. The strains are listed in Table 1. Some were freshly isolated but the majority were received from other workers and from culture collections. Each strain was given a computer reference number but the original strain designation is given in column 2 of Table 1. The strains were originally classified by one of us (D.J.) in collaboration with the late P.M.F. Shattock on the basis of criteria now published by Jones & Shattock (1960), Smith & Shattock (1962), Deibel, Lake & Niven (1963) and Whittenbury (1965). Where known, the source of every strain is listed and also the literature reference if the strain has appeared in published work. The strains were lyophilized and working cultures were maintained on a medium containing 1.0% (w/v) peptone, 1.0% (w/v) Lab Lemco, 0.5% (w/v) NaCl, 0.5% (w/v) glucose.

Tests. The tests are listed in Table 2. The basal medium for the tests, unless otherwise stated, was as described above, but with the glucose omitted.

Numerical analysis. The results of 45 tests performed on the 122 strains were analysed by J. W. Carmichael's computer program 'Taxon'. Like most procedures for numerical taxonomy this method proceeds in two stages: computation of a matrix of similarity between all pairs of OTUs (Operational Taxonomic Units, i.e. the objects being classified) and the formation of clusters from the similarity matrix. Unlike most numerical taxonomic procedures the classification is not in general hierarchical. Therefore the clusters cannot normally be represented by a dendrogram. However, the method provides extensive information on the consistency of the groups and on their interrelations, and this information is readily amenable to two-dimensional diagrammatic representation in forms which are more informative than a dendrogram.

One may represent the OTUs as points in an n -dimensional attribute space, where n is the number of attributes. The method is an attempt to extend to many dimensions the intuitive processes of cluster formation among configurations of points in two dimensions. In this description the terms 'OTU', 'point', 'member' and 'strain' are used more or less interchangeably. Carmichael, Julius & Martin (1965) have described the rationale behind the choice of similarity coefficient, and Carmichael, George & Julius (1968) have described the background and details of the clustering method, and an example on bacteria is given in Carmichael & Sneath (1969).

Most of the attributes in this study were two-state test results with values (states) coded 0 (negative reaction) and 1 (positive reaction), but for several tests three states could be recognized: negative (coded as 0), slow positive reaction (1), and fast positive reaction (2). These attributes contain more information than the two-state attributes; the program accordingly gives them more weight in the computation of the similarity coefficients. Each

Table 1. *Strains used in study and their source where known*

Computer listing	Strain	Source	Reference†
<i>Streptococcus faecalis</i>			
1	370	—	—
2	DS/753	—	—
3	H69D5	—	Lancefield (1941)
4	NCTC775	—	Working type, Sneath & Skerman (1966)
5	B8	Infant faeces	} Sharpe & Shattock (1952)
6	N83	Infant faeces	
7	S161	Infant faeces	
8	NCTC6782	—	—
9	S41	Silage	Whittenbury (1965)
<i>S. faecalis</i> var. <i>zymogenes</i>			
10	Magill	—	—
11	D76	—	Lancefield (1941)
12	N37	Infant faeces	Sharpe & Shattock (1952)
<i>S. faecalis</i> var. <i>liquefaciens</i>			
13	Elv2025	—	—
14	GB122	Infant faeces	} Sharpe & Shattock (1952)
15	N97	Infant faeces	
<i>S. faecium</i>			
16	H2678	Gruyère cheese	Jones & Shattock (1960)
17	H2766/1	Canned ham	—
18	H2766/2	Canned ham	—
19	H2767	Canned ham	—
20	P5	Pig intestine	Jones & Shattock (1960)
21	P/1/3	} Canned ham	—
22	P/5/3		
23	P/6/1		
24	P/7/1		
25	US7		
26	HGH9		
27	HGH13		
28	HGH421		
29	HGH511	—	Sharpe & Fewins (1960)
30	1 MEC*	River water	—
31	2 MEC*	River water	—
32	3 MEC*	River water	—
33	4 MEC*	River water	Jones & Shattock (1960)
34	5 MEC*	River water	—
35	6 MEC*	River water	—
36	1*	Silage	—
37	32*	Silage	—
38	60*	Silage	Jones & Shattock (1960)
39	73*	Silage	—
40	S748*	Human endocarditis	Jones & Shattock (1960)
<i>S. durans</i>			
41	98D (NCTC8307)	—	Type strain, Sneath & Skerman (1966)
42	Cu 2*	Canned ham	—
43	SD21*	Canned ham	—
44	Arg 117	—	—
45	H2	Infant faeces	} Sharpe & Shattock (1952)
46	N16	Infant faeces	
47	Hirsch 503	Pasteurized milk	—
48	Hirsch 613	Pasteurized milk	—
49	Mocquot I, 1	Roquefort cheese	—
50	Mocquot I, 2	Roquefort cheese	—
51	Hiscox F2	Cheddar cheese	—

Table 1 (*cont.*)

Computer listing	Strain	Source	Reference†
52	Hiscox H20	Cheddar cheese	—
53	498SDA (NCDO498)	—	—
54	Czulak SD1	—	—
55	Czulak SD2	—	—
56	NF/12 (Niven)	—	—
57	NF/13 (Niven)	—	—
58	NF/3 (Niven)	—	—
59	A2 (Niven)	—	—
60	S5*	Raw milk	—
61	17/C1	Human faeces	—
<i>S. bovis</i>			
62	Pearl 11	Cow dung	Shattock (1949)
63	Lottie 15	Cow dung	—
64	Peach 23	Cow dung	—
65	Campion 11	Cow dung	Shattock (1949)
66	S182	Infant faeces	—
67	S112	Infant faeces	Sharpe & Shattock (1952)
68	S194	Infant faeces	—
69	Rosie 10	Cow dung	—
70	Rosalie 20	Cow dung	Shattock (1949)
71	BS07	Sheep rumen	Macpherson (1953)
72	16B	Sheep rumen	—
73	25A	Sheep rumen	—
74	18/M1	Cow rumen	} Bailey & Oxford (1958)
75	18/M2	Cow rumen	
76	18/C2	Cow rumen	
77	19/C1	Cow rumen	—
78	17/M4	Cow rumen	} Bailey & Oxford (1958)
79	22/M2	Cow rumen	
80	24/M4	Cow rumen	
81	63	Cow rumen	—
82	64	Cow rumen	Higginbottom & Wheeler (1954)
83	65	Cow rumen	—
84	67	Cow rumen	—
85	L5	Cow	—
86	L8	Cow	—
87	L21	Cow	—
88	L23	Cow	—
89	L24	Cow	Perry, Wilson, Newland & Briggs (1955)
90	L25	Cow	—
91	S11	Cow	—
92	S16	Cow	—
93	S18	Cow	—
94	S24	Cow	—
95	S25	Cow	—
96	S26	Cow	—
97	S29	Cow	—
98	S33	Cow	—
Unclassified			
99	S39	Cow	—
<i>S. bovis</i>			
100	W5	Cow	—
101	W6	Cow	—
102	574*	Human endocarditis	—
103	79*	Raw milk	—
104	156*	Raw milk	—
105	165*	Raw milk	—
106	166*	Raw milk	—

Table 1 (cont.)

Computer listing	Strain	Source	Reference†
		Serological group Q	
107	E6556	—	Guthof (1955)
108	E6749	—	
109	E6844	—	
		<i>S. equinus</i>	
110	Susan 1*	Horse manure	—
111	Susan 2*		
112	Susan 3*		
113	Tinker 1*		
114	Tinker 2*		
115	Tinker 3*		
116	Umpire 1*		
117	Umpire 2*		
118	Umpire 3*		
119	Unicorn 1*		
120	Unicorn 2*		
		<i>S. faecalis</i> var. <i>malodoratus</i>	
121	864	Gouda cheese	—
122	847	Gouda cheese	—

NCTC = National Collection of Type Cultures.

* Strains freshly isolated at time of study.

† Published work citing particular strains.

Table 2. *The morphological, physiological and biochemical tests used in the study*

Gram stain

Cellular morphology: coccoid; pleomorphic

 β -Haemolysis in horse blood agar (Brown, 1919)

Serological grouping: Lancefield groups D and Q (Jones & Shattock, 1960)

Survival after heating 60 °C for 30 min

Growth at 10 °C, 45 °C, 50 °C

Growth at pH 9.6 (Shattock & Hirsch, 1947)

Growth in NaCl (6.5 %, w/v) nutrient broth

Growth on bile (40 %, w/v) horse blood (5 %, v/v) agar

Growth on potassium tellurite (0.04 %, w/v) horse blood (5 %, v/v) agar

Growth on sodium azide (0.02 %, w/v) nutrient agar

Growth on thallous acetate (0.02 %, w/v) nutrient agar

Reduction of 2,3,5-triphenyltetrazolium chloride (Barnes, 1956)

Growth in litmus milk medium: reduction of litmus before clotting; digestion of acid clot

Gelatin liquefaction

Ammonia from arginine (Niven, Smiley & Sherman, 1942)

Hydrolysis of starch (aerobic)

Hydrolysis of starch (anaerobic)

Hydrolysis of sodium hippurate (Ayers & Rupp, 1922; ferric chloride reagent)

Production of acid from fermentable substances (bromocresol purple indicator): glucose, maltose, sucrose, lactose, cellobiose, trehalose, arabinose, melibiose, melezitose, raffinose, glycerol (aerobic), glycerol (anaerobic), mannitol, sorbitol, inulin, salicin, aesculin

Catalase activity

Utilization of citrate as energy source (Deibel, Lake & Niven, 1963)

Utilization of malate as energy source (Deibel, Lake & Niven, 1963)

Decarboxylation of malate (Whittenbury, 1965)

Production of hydrogen sulphide

attribute is given a weight of $\log_2(s)$, where s is the number of states. Thus the two-state attributes had unit weight and the three-state attributes had a weight of 1.585 ($\log_2(3) = 1.585$, approximately). Three attributes were constant over all 122 strains. The program assigned them each a weight of zero and they were thereby effectively ignored. The similarity measure was that described in the appendix to Carmichael & Sneath (1969).

The program then goes on to the clustering. A cluster is here thought of as a group of densely packed points surrounded by a region of empty space or of thinly scattered points. A cluster is built up by the successive addition of points until no point sufficiently close to the cluster can be found. The closest pair of points form the initial members of the first cluster. Subsequent clusters start formation with the closest pair of points not already in a cluster. When no sufficiently close pair is found clustering is complete. Any points not placed in a cluster remain as one-member clusters.

The whole clustering process is repeated at successively lower 'levels of resolution' (there were 12 in all). At each successive level the conditions of insufficient closeness for points to join clusters are relaxed.

At all stages in the formation of a cluster the next OTU to join is the closest point to any member of the cluster. It is, however, prevented from joining if it contravenes any of four conditions given by Carmichael *et al.* (1968). The cluster is then complete and the program tries to start a new cluster. Any point which has not been placed in a cluster but whose nearest neighbour is in a cluster is described as a satellite of the cluster.

The output from the program allows convenient and detailed two-dimensional representation of the classified OTUs. In this study we followed the practice of Carmichael & Sneath (1969) and drew some of the clusters as circles whose diameters are proportional to the distance between the most unrelated pair of OTUs in the cluster. We gave prominence to the most stable clusters, i.e. those which remain unchanged through several successive levels of resolution. We also showed some degree of hierarchy in the clusters, from comparisons of the results at several levels of resolution.

At the highest levels of resolution the formation of many of the very tight groups was halted simply by their becoming too disperse. Moreover, since there were many ties or near-ties among the highest similarity values, the results at these resolution levels were often dependent on the order of the OTUs in the data and the results of just one or two discriminatory tests. However, these factors mainly affect details within the main clusters and do not greatly affect the taxonomic conclusions.

The results of the Carmichael analysis were compared with an analysis of part of the data (38 tests on 119 strains) by G. J. S. Ross's program CLASP. This program computed coefficients of S_a (Gower, 1971) in which matching zeros in the two-state characters are excluded from the comparisons, and clustering was by single linkage (Sokal & Sneath, 1963).

RESULTS

The majority of the 122 strains clustered into five groups which were most stable at resolution 5 (average link parameter 0.763, single link parameter 0.852; see Carmichael *et al.* 1968). The results are depicted graphically in Fig. 1. This Figure is based on information derived mainly from resolution 5 with additional information from other levels of resolution where this was required to fix more accurately the relationships of strains to one another. All the strains are included in the Figure but in the interests of clarity not all are labelled. Several strains were identical and in these cases only one point is marked.

Group 1. This group contains all the strains identified as *Streptococcus faecalis*, *S. faecalis*

Fig. 1. Two-dimensional representation along two distance axes (X, Y) of the clustering of 122 strains of streptococci of serological group D. ○, *Streptococcus faecalis* var. *faecalis*; ⊕, *S. faecalis* var. *liquefaciens*; ●, *S. faecalis* var. *zymogenes*; △, *S. faecium*; ▲, *S. durans*; +, serological group Q; ▼, *S. equinus*; □, *S. bovis*; ■, unclassified. ~~, Distance between strains 3 and 48 = 0.2 units; ~ distance between strains 28 and 103 = 0.15 units.

var. *liquefaciens* and *S. faecalis* var. *zymogenes*. At resolution 1 (average link parameter 0.940, single link parameter 0.940) two separate clusters were formed. One contained six strains of *S. faecalis* with strains 1, 4 and 9 as satellites, and the other contained two strains of *S. faecalis* var. *liquefaciens* and two strains of *S. faecalis* var. *zymogenes* with strains 10 and 14 as satellites. At the next level of resolution only strains 9 and 10 remained as satellites of the main cluster and at resolution 3 (average link parameter 0.852, single link parameter 0.896) all strains of *S. faecalis*, *S. faecalis* var. *liquefaciens* and *S. faecalis* var. *zymogenes* grouped together and remained separate from all the other strains in the study at all subsequent levels of resolution including the last (average link parameter 0.454, single link

parameter 0.697). The two strains (121 and 122) received as *S. faecalis* var. *malodoratus* did not cluster with Group 1 nor were they related to this group (see below).

Group 2. All the strains designated *Streptococcus faecium* and *S. durans* are included in this group. At all resolutions up to 5 the strains in this group formed several clusters which were not obviously related to source of strains nor to species name. Even at resolution 5 four strains of *S. durans* (41, 42, 43 and 44) and one strain of *S. faecium* (24) remained as satellites of the main cluster. This situation remained at resolution 6 (average link parameter 0.719, single link parameter 0.830) but, after this, all strains of *S. faecium* and *S. durans* merged to form one group until resolution 10 when all the strains of *S. avium* (Group 3) joined the *S. faecium*-*S. durans* complex.

Group 3. All the strains of *Streptococcus avium* clustered together at resolution 1 (average link parameter 0.940, single link parameter 0.940) and remained as a tight cluster until an unclassified strain (99) joined at resolution 9 (average link parameter 0.587, single link parameter 0.763). At the next resolution all, including the unclassified strain, merged with the *S. faecium*-*S. durans* complex.

Group 4. The majority of the strains identified as *Streptococcus equinus* (and found to be in close accord with the original description of Andrewes & Horder, 1906) formed a cluster at resolution 1 (average link parameter 0.940, single link parameter 0.940) and remained distinct until they were merged with the majority of the *S. bovis* strains at resolution 6 (average link parameter 0.719, single link parameter 0.830). *Streptococcus equinus* strain 111 always clustered with the *S. bovis* strains.

Group 5. This group comprises the majority of strains identified as *Streptococcus bovis* and, as mentioned above, *S. equinus* strain 111. At resolutions 1 to 3 several small groups of *S. bovis* clustered together but there was no connexion between the groupings and source of strains nor did the mannitol-fermenting strains of *S. bovis* group together. At resolution 4 (average link parameter 0.808, single link parameter 0.874) all the strains of *S. bovis* (except the satellites 102 and 104) and also *S. equinus* strain 111, clustered together and remained as one group until resolution 6. At this level all the strains of *S. equinus* and *S. bovis* (except 102 and 104 which remained as satellites) merged.

Ungrouped strains. Six strains did not enter any of the five major groups. With the exception of *Streptococcus faecalis* var. *malodoratus* (two strains), which were probably closest to *S. avium* but did not form a satellite of this group, the other strains were close satellites of one of the main groups. Strains 102 and 104 were both satellites of Group 5 as noted above, and because of the limitations of two-dimensional representation strain 102 appears to be contained within Group 5 in the diagram. One unclassified strain (65) is also a satellite of Group 5 and the other (99) a satellite of Group 3.

DISCUSSION

The majority of the strains clustered around five phenetic nodes, *Streptococcus faecalis* and its varieties, *S. faecium*-*S. durans*, *S. avium*, *S. bovis* and *S. equinus*.

The grouping of *Streptococcus faecalis* and its varieties *S. faecalis* var. *liquefaciens* and *S. faecalis* var. *zymogenes* into one cluster with little evidence of any internal grouping strengthens the case made by Deibel (1964) for dropping the varietal epithets '*liquefaciens*' and '*zymogenes*' and regarding these varieties as functional biotypes of *S. faecalis*.

The other strains in the enterococcus division as defined by Sherman (1937) all clustered together in one rather diffuse group which we refer to as *Streptococcus faecium*-*S. durans*. The elongation of this group is due largely to four strains of *S. durans* (41, 42, 43 and 44).

Two of these strains (41 and 44) are strains on which Sherman & Wing (1935, 1937) based the description of the species. We do not think a case can be made for the retention of the separate species names *S. faecium* and *S. durans*. Deibel *et al.* (1963) and Whittenbury (1965) have also considered *S. durans* to be a variety of *S. faecium*. Similarly, a close relationship between *S. faecium* and *S. durans* and some separation of this complex from *S. faecalis* strains is seen in the numerical study of Colobert & Blondeau (1962). In those numerical studies where *S. durans* has appeared to be separate from *S. faecium* very few strains of *S. durans* were included. Colman (1968) used one strain, Raj & Colwell (1966) used four strains and Seyfried (1968) used two strains of *S. durans*. In each case, one of the strains used was strain 41 of this study. In the present study 21 strains identified as *S. durans* were studied. These included the type strain (41, SHERMAN98D, NCTC8307) and other old laboratory strains as well as a number of strains isolated from cheese, raw and pasteurized milk, canned hams and human faeces. Since we do not think there is sufficient reason for retaining the two species epithets '*faecium*' and '*durans*', *S. faecium* Orla-Jensen (1919) has precedence.

The separate species status given to strains possessing the group Q antigen (*Streptococcus avium*) by Nowlan & Deibel (1967) is borne out by the results of the present study, though only three strains are included. The results also agree with the numerical study of Colman (1968) in showing a close relationship between *S. avium* and *S. faecium* though not close enough, in our estimation, for *S. avium* to be regarded as a biotype of *S. faecium*.

A close relationship was noted between *Streptococcus bovis* and *S. equinus*. This is in agreement with the results of Seeley & Dain (1960) and Smith & Shattock (1962) but contrary to the results of Raj & Colwell (1966), who based their opinion on the reactions of one strain of *S. equinus* (ATCC9812) which, in their study, showed a closer relationship to the pyogenic streptococci than to *S. bovis*. It is, however, not yet clear how far this strain (which has not been widely studied by streptococcus workers) is representative of Andrewes & Horder's species *S. equinus*. They also suggested the inclusion of *S. bovis* in the enterococcus division of the streptococci even though *S. bovis* does not conform with all the criteria used by Sherman (1937) to define this division. In the present study *S. bovis* and *S. equinus* join the *S. faecium*-*S. durans* complex at resolution 11 (average link parameter 0.498, single link parameter 0.719) this is a relatively low level of similarity in a group where recent genetic studies have further emphasized the homogeneity first stressed by Andrewes (1906).

Within the species *Streptococcus bovis* there is no indication of two biotypes as suggested by Medrek & Barnes (1962). The characters which these authors suggested might effect this separation were: production of acid from mannitol, reaction on horse blood agar and ability to produce slime on a sucrose gelatin medium. The first two, but not the last test, were included in the present study. In our experience strains designated *S. faecium* as well as *S. bovis* can produce slime on a sucrose gelatin medium (D. Jones, unpublished), and later work by Barnes, Seeley & Van Demark (1961) did not confirm the close correlation (noted in the earlier work of Dain *et al.* 1956) between ability to ferment mannitol and inability to produce slime from sucrose by *S. bovis*.

The result of any classification of a group of organisms is dependent on the selection of strains, the choice of characters and, in the case of numerical studies, on the choice of computer program. The strains used in this study were isolated from a variety of habitats in a number of different countries and were assembled because, at the time of study, the laboratory in which one of us worked acted as a reference laboratory for the serological group D. A relatively small number of characters were used, but they included those

recommended by other workers (Deibel, 1964; Whittenbury, 1965) to effect a separation within the faecal streptococci. Only two morphological characters were used, because in our experience detecting morphological differences in such a homogeneous group as the streptococci is apt to be a highly subjective procedure, and we were only confident of the reproducibility of these two.

The 'Taxon' program used in this study provides information which can be represented in the form of a two-dimensional figure, but it can be criticized because the clusters can be limited by one OTU blocking the entrance of others into a particular cluster. For example, in the present study, Group 1 (*Streptococcus faecalis*) remains separate from all the other groups at all levels of resolution. Is this because its 'nearest neighbour', strain 48, is not sufficiently similar to enter the group and therefore blocks the entry of other members of the *S. faecium*-*S. durans* complex? Apparently not, because a minimal spanning tree (Ross, 1969) kindly computed by G. J. S. Ross at Rothamsted Experimental Station showed groups in very close accord with the ones from 'Taxon', as did a principal co-ordinates analysis. A phenogram based on single link clustering was also in good accord with all of these.

The results of the analyses suggest that the variation between *Streptococcus faecium* and *S. durans* is almost continuous (although they span a large distance in phenetic space). It seems likely from these rather limited data that fewer intermediates occur, if any, between *S. faecalis* and the *S. faecium*-*S. durans* complex, than between this complex and *S. bovis*.

In conclusion, on the basis of this study, five groups of streptococci of serological group D, *Streptococcus faecalis*, *S. faecium*, *S. avium*, *S. bovis* and *S. equinus* seem sufficiently distinct and well defined to warrant separate species status. There is, however, a need for a new look at the classification of the streptococci as a whole group. Such a study should include adequate representation, not just single strains, of all species representing all four divisions of Sherman's (1937) groupings.

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