Numerical Taxonomy of Genera *Micrococcus* Cohn and *Sarcina* Goodsir

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SUMMARY

A group of 62 strains, mostly members of the genera *Micrococcus* and *Sarcina*, has been subjected to a numerical-taxonomic analysis. All 57 characters have been scored according to the scale 0 to 5 and an appropriate formula has been used for the calculation of similarity. The method of clustering of organisms applied here is a modification of the original 'single-linkage method'. Eleven 85-phenons were found and the rank of species has been attributed to them. On the basis of existing taxonomic studies it appears probable that aerobic sarcinas must be considered as members of the genus *Micrococcus* and that they are R-dissociated forms of proteolytic micrococci.

INTRODUCTION

The extensive literature existing today on the taxonomy of micrococci includes such comprehensive papers as those by Abd-el-Malek & Gibson (1948), Shaw, Stitt & Cowan (1951), Kocur & Martinec (1962), Baird-Parker (1963, 1965). The numerical taxonomy introduced in the classification of bacteria by Sneath (1957*a, b*) and broadly used in the present taxonomy of micro-organisms, was for the first time applied to micrococci by Hill (1959) and later by Pohja (1960), Pohja & Gyllenberg (1962), Hubálek (1964), Hill *et al.* (1965). The results obtained in the application of numerical taxonomy were compared with the ratio of DNA bases in this group of bacteria by Silvestri & Hill (1965) and Rosypal, Rosypalová & Hořejš (1966). This communication is based on an earlier study (Hubálek, 1964) and applies the modified Sneath's method to non-pathogenic micrococci and sarcinas.

METHODS

A total of 62 strains of the genera *Micrococcus* Cohn, *Sarcina* Goodsir, and *Staphylococcus* Rosenbach from the Czechoslovak Collection of Micro-organisms (CCM) have been studied and a list of them is presented in Table 1. All the tests were performed two or three times and in the same way as by Kocur & Martinec (1962) unless otherwise indicated. The cultivation temperature was 30° and both a 'positive' culture and a negative control in each test were used for comparison. A complete description of the methods used has been given by Hubálek (1964).

Characters

The final set of 57 characters does not comprise 'unsuitable' ones, i.e. those which in all strains were negative (e.g. acetoin and indole production, benzoate utilization, phenylalanine decomposition and presence of lecithinase), inconstant or difficult to classify (milk decomposition, colony profile and margin).

All characters have been evaluated according to the scoring scale of 0 to 5 with the degrees 0, 1, 2, 3, 4, 5 (using intergrades of 0.5) corresponding to the increasing intensity of character manifestation. The degree 0 represents the absence of a character, the degree 5 its highest intensity. The same scoring system has been used by Poncet (1967).

Table 1. *Test organisms*

Original names	CCM numbers
<i>Micrococcus citreus</i>	105, 642, 644, 683 (ATCC 9510), 848
<i>M. cyaneus</i>	852
<i>M. epidermidis</i>	253, 257
<i>M. flavus</i>	210 (ATCC 400), 291, 531, 546, 555 (ATCC 10240 a), 617, 732 (ATCC 10240), 819, 1424 (NCTC 7743), 1681
<i>M. luteus</i>	294, 810 (ATCC 398), 824
<i>M. lysodeikticus</i>	169 (NCTC 2665), 276, 530, 550, 656, 1335, 1423 (ATCC 12698), 1680
<i>M. sodonensis</i>	144 (ATCC 11880)
<i>Micrococcus</i> sp.	46, 52, 80, 134, 135, 183, 661,
<i>Sarcina citrea</i>	248, 631
<i>S. flava</i>	163, 166, 296, 308, 335, 351 (ATCC 540), 410 (ATCC 272)
<i>S. lutea</i>	149, 249, 310, 336, 337 (ATCC 382), 357, 362, 407, 443, 523, 536
<i>S. marginata</i>	265
<i>S. subflava</i>	209
<i>S. variabilis</i>	266
<i>Staphylococcus citreus</i>	688
<i>S. flavocyaneus</i>	247 (NCTC 7011, ATCC 8676)

Survey of characters

1. *Gram reaction*. The preparations were made from meat-peptone agar (MPA) and meat-peptone broth (MPB) from 20-, 45- and 115-hr-old cultures. From each strain six preparations were made, stained, and the Gram reaction has been calculated as the arithmetic mean of all these values and rounded to the nearest degree of the scoring scale.

Scoring: 0, typically Gram-negative; 1, Gram-negative; 2, Gram-variable, tending to Gram-negative; 3, Gram-variable, tending to Gram-positive; 4, Gram-positive; 5, strongly Gram-positive.

2. *Occurrence of packets in preparations from MPA*. Average value of three wet-mount preparations (1-, 2- and 5-day-old cultures).

Scoring: 0, typical grape-like clusters; 1, less typical grape-like clusters; 2, small clusters, less abundant tetrads and also short chains; 3, prevailing tetrads; 4, typical tetrads, less abundant octads; 5, packets of minimal number of 16/32 cells.

3. *Occurrence of packets in preparations from MPB*. Scoring as character 2. The score was different in some strains from that in character 2, therefore the character has been included.

4. *'Membranes'*. In preparations of some strains (CCM 294, 824, 848) membranes

surrounding several cocci have been found. Scoring 0 to 5 according to occurrence frequency of this structure, by mutual comparison of the strains.

5. '*Compressed tetrads*'. The cells of some strains (CCM 253, 257, 247, 852) appeared constantly to be just dividing into tetrads; the whole grouping looked like a very 'compressed' tetrad. Scoring 0 to 5, analogically as character 4.

6. *Turbidity in MPB*. Estimated at the 1st, 2nd and 5th day, from these values the arithmetic mean has been calculated and rounded to the nearest degree of the scoring scale.

Scoring: 0, none; 1, weak; 2, rather weak; 3, moderate; 4, dense; 5, very dense.

7. *Conglomerates in MPB*. In liquid medium typical sarcinas produce small conglomerates, often visible by naked eye. Evaluation was made on the 5th day by means of a $\times 6$ hand lens.

Scoring: 0, homogenous turbidity; 1, very fine conglomerates hardly discernible by lens; 2, rather fine conglomerates visible only by lens; 3, moderately large conglomerates scarcely visible by naked eye; 4, large conglomerates visible by naked eye; 5, very conspicuous conglomerates easily visible by naked eye.

8. *Ring pellicle in MPB*. The highest value attained within 5 days.

Scoring: 0, none; 1, a trace on the wall of the test-tube; 2, slight, disconnected; 3, medium, uninterrupted or disconnected; 4, strong, consistent; 5, very strong.

9. *Intensity of sediment in MPB*. Average value from data on the 1st, 2nd and 5th day.

Scoring: 0, slight or none; 1, weak; 2, rather weak; 3, medium; 4, strong; 5, very strong.

10. *Character of sediment in MPB*. Evaluated on the 5th day according to the following scheme:

Presence of viticula	Dispersion of sediment on shaking	Appearance and character of sediment	Scoring
$\pm$	—	Mucous	0
+	—	Viscid	1
+	$\pm$	Viscid	2
$\pm$	+	Slightly granular	3
—	+	Granular	4
—	++	Strongly granular	5

Graded response: ++, +, $\pm$, —.

The term 'viticula' designates a corkscrew-like formation of sediment created on the bottom of the test-tube after careful rotatory shaking.

11. *Transparency of colony*. From 3-day cultures on MPA.

Scoring: 0, very opaque; 1, opaque; 2, slightly translucent; 3, translucent; 4, slightly transparent; 5, clearly transparent.

12. *Surface roughness of colony*. The data are average values obtained after 5-day cultivation on MPA and glucose yeast-extract agar.

Scoring: 0, surface mucous, very glossy; 1, glossy; 2, less glossy; 3, matt; 4, strikingly matt to granular; 5, strongly granular.

13. *Yellow pigmentation of colony*. Inoculated MPA slants were incubated for 2 days in thermostat at 30° and then kept at room temperature in diffuse light for 5 days. The evaluation is the average value obtained from several repeated observations.

Scoring: 0, colourless; 1, white, whitish or greyish; 2, slightly yellowish; 3, pale lemon; 4, lemon to yolk yellow; 5, pure yolk yellow.

14. *The highest grade of yellow pigment.* Seen on a number of solid media. In several strains the pigmentation on media such as glucose yeast-extract agar or starch agar was deeper than on the MPA. Scoring as character 13.

15. *Orange pigment on MPA.* Scoring 0 to 5 according to intensity. Estimated by mutual comparison of all the cultures.

16. *Brown exopigment on MPA.* Scoring as for character 15.

17. *Violet exopigment on glucose yeast-extract agar.* Scoring as for character 15.

18. *Colony diameter on MPA.* An inoculum from a streak culture was inoculated onto three spots on one 15 ml. MPA plate in a 10 cm. Petri dish; after 48-hr cultivation the diameter of the three colonies was measured and the arithmetic mean of these values calculated for each strain.

Scoring: 0, no growth, never seen; 1, diam. 1 mm.; 2, diam. 2 mm.; 3, diam. 3 mm.; 4, diam. 4 mm.; 5, diam. 5 and more mm.

19. *Optimal temperature.* Determined on MPA.

Scoring: 0, about 18 to 20°; 1, about 21 to 23°; 2, about 24 to 27°; 3, about 28 to 31°; 4, about 32 to 35°; 5, about 36° and more.

20. *NaCl tolerance.* Determined on MPA with various concentrations of salt.

Scoring	5 % NaCl	7.5 % NaCl	10 % NaCl	12.5 % NaCl
0	—	—	—	—
1	±	—	—	—
2	+	±	—	—
3	+	+	±	—
4	+	+	+	±
5	+	+	+	+

Legend: +, growth; ±, weak growth; —, no growth.

21. *Aesculin hydrolysis.*

Scoring: 0, medium without colour change; 1, a trace of brown coloration within 25 days; 2, brown coloration within 14 days; 3, black coloration in 6 to 14 days; 4, black coloration in 2 to 5 days; 5, black coloration within 1 day.

22. *Starch hydrolysis.* After 5-day cultivation the breadth of zone with degraded starch was measured.

Scoring: 0, no zone; 1, zone 0.5–1.0 mm.; 2, zone 1.5–2.5 mm.; 3, zone 3.0–4.5 mm.; 4, zone 5.0–10.5 mm.; 5, zone 11.0 mm. and more.

23. *Glucose 'oxidation'* (formation of acids in Hugh–Leifson medium with glucose and bromthymol-blue).

Scoring: 0, without colour change of indicator in 30 days; 1, green-yellow coloration of indicator in 30 days; 2, yellow coloration of indicator not before 15 days; 3, yellow coloration of indicator in 10 to 14 days; 4, yellow coloration of indicator in 5 to 9 days; 5, yellow coloration of indicator in 1 to 4 days.

24. *Glucose 'fermentation'* (formation of acids in Hugh–Leifson medium, over which a 2 cm. column of liquid paraffin was poured). Scoring as character 23.

25. *Methyl red test.* 1-, 3-, 5- and 7-day-old cultures were tested in each strain.

Scoring: Colour of the indicator added—0, yellow in all the cultures; 1, very slight

orange at most; 2, orange at most; 3, red not before the 5th day; 4, red in a 3-day culture; 5, red in a 1-day culture.

26. *Gluconate oxidation and growth in the medium.* None of the strains used oxidized gluconate; growth was scored after 10 days.

Scoring: 0, no growth; 1, weak growth; 2, good growth. Degrees 3 to 5 would correspond to positive biochemical reactions which were not found in the set of the strains under investigation.

27 to 34. *Utilization of salts of organic acids.* Concentrations (% w/v): sodium acetate 0.2, sodium lactate 1.0, sodium potassium tartrate 1.0, ammonium tartrate 0.5, sodium formate 0.1, ammonium succinate 1.0, sodium citrate 0.5, sodium citrate 0.5 in the presence of Difco-yeast extract 0.01.

Scoring: 0, without colour change of indicator within 30 days and not growing; 1, without colour change of indicator or trace—growing weakly; 2, without colour change of indicator or trace—growing well; 3, colour change of indicator in 15 to 20 days; 4, colour change of indicator in 7 to 14 days; 5, colour change of indicator in 2 to 6 days.

35. *Hucker's test.* (Utilization of $\text{NH}_4\text{H}_2\text{PO}_4$ as a sole source of nitrogen in the presence of glucose.) Scoring as characters 27 to 34.

36. *Hucker's test, with Difco-yeast extract (0.01 %) in medium.* Scoring as characters 27 to 34.

37. *Tyrosine oxidation.* Scoring as character 26.

38. *Gelatin hydrolysis.* After 3-day incubation the diameters of the hydrolytic zones round the colonies on plates of MPA plus 10% gelatin after pouring on HgCl_2 reagent were measured. Scoring as character 22.

39. *Halo-zones on MPA.* After inoculating a thin MPA layer in a Petri dish in a straight streak, some strains show turbid zones probably due to the activity of proteolytic enzymes. Scoring as character 22.

40. *Casein hydrolysis.* Read after 3 days; scoring as character 22.

41. *Proteolytic-lipolytic activity in egg agar.* Scoring 0 to 5 by comparison of cultures.

42. *H_2S production.* This was determined by a rather sensitive microtest (MPB plus 0.1% sodium thiosulphate, with a suspended lead acetate paper). Scoring according to the amount of PbS produced, estimated by visual comparison of the indicator papers.

43, 44. *Lysine and arginine decarboxylation.*

Scoring: 0, without colour change of indicator in 50 days; 1, colour change of indicator not before 40 days; 2, colour change of indicator in 30 to 39 days; 3, colour change of indicator in 15 to 29 days; 4, colour change of indicator in 5 to 14 days; 5, colour change of indicator in 1 to 4 days.

45. *Haemolysis of sheep red blood corpuscles.*

Scoring: 0, no zone in 5 days; 1, a slight trace of zone in 5 days; 2, a well discernible trace in 5 days; 3, a distinct transparent zone in 4 to 5 days; 4, a distinct transparent zone in 3 days; 5, a zone in 1 to 2 days, never seen.

46. *Urea decomposition.* Studied by two methods: the resulting value is the average of both.

(a) Microtest after Cowan.

Scoring: 0, without colour change of indicator in 40 hr; 1, orange coloration of indicator in 30 to 40 hr; 2, orange coloration of indicator within 29 hr; 3, red

coloration of indicator not before 16 hr; 4, red coloration of indicator in 9 to 15 hr; 5, red coloration of indicator within 8 hr.

(b) The test with liquid medium. Concentration (% w/v): urea 2.0, KH_2PO_4 0.0091, $(\text{NH}_4)_2\text{HPO}_4$ 0.0095, Difco-yeast extract 0.01, phenol red 0.001.

Scoring: 0, without colour change of indicator and not growing; 1, without colour change of indicator, growing; 2, colour change of indicator in 5 to 14 days; 3, colour change of indicator in 2 to 4 days; 4, colour change of indicator in 13 to 24 hr; 5, colour change of indicator in 12 hr.

47. *Nitrate reduction* (0.05% KNO_3). Read after 5 days incubation, after the addition of Griess–Illosvay reagent.

Scoring: 0, no reduction; 1, medium slightly reddish; 2, medium weakly reddish; 3, medium red; 4, red-brown precipitate in medium; 5, very intensive tufts of red-brown precipitate or simultaneous absence of NO_2^- and NO_3^- as demonstrated by means of zinc powder.

48. *Nitrite decomposition*. Semisolid medium with 0.0005% NaNO_2 . 2-, 5- and 7-day cultures were tested in each strain.

Scoring: Colour of medium after addition of Griess–Illosvay reagent— 0, deeply red in each culture (2, 5, 7 day); 1, red; 2, faintly red at most; 3, without colour change not before the 7th day; 4, without colour change in a 5-day-old culture; 5, without colour change in a 2-day-old culture.

49. *Methylene-blue reduction*. Scoring 0 to 5 by comparison of cultures.

50. *Catalase production*. Scoring 0 to 5 by comparison of cultures.

51 to 55. *Sensitivity to antibiotics*. The test was performed on MPA plates with 'Spofa' test tablets: penicillin 10 i.u., chloramphenicol 20 μg ., neomycin 40 μg ., nystatin 20 i.u., bacitracin 5 i.u. per tablet.

Scoring: 0, inhibition zone 0 to 1 mm.; 1, inhibition zone 1.5 to 2 mm.; 2, inhibition zone 2.5 to 6 mm.; 3, inhibition zone 6.5 to 11 mm.; 4, inhibition zone 11.5 to 16 mm.; 5, inhibition zone 16.5 mm and more.

56. *Sensitivity to lysozyme*. Lysozyme solution was placed in wells in inoculated agar plates (about 20 μg . of the substance per well). Scoring as for characters 51 to 55.

57. *Sensitivity to N-6 phage* (host strain is *Micrococcus lysodeikticus* CCM 1335). One drop of phage preparation was placed on a lawn on MPA plates.

Scoring: 0, growth unaffected; 1, a trace of weaker growth; 2, distinctly weaker growth; 3, translucent plaque in the spot covered by drop, but with more than 50 microcolonies in it; 4, 6 to 50 microcolonies in plaque; 5, 0 to 5 microcolonies in plaque. This evaluation is a rough expression of the frequency of sensitive cells in the population.

Numerical methods

In contrast to the existing most usual formula for calculating similarity (Sneath, 1957b):

$$S = \frac{n_s}{n_s + n_d},$$

where n_s = number of characters possessed by both individuals, n_d = number of different characters, another formula has been applied (Hubálek, 1964), in order to use the scale of 0 to 5, in scoring characters:

$$S = \frac{r \cdot \Sigma p - \Sigma |d|}{r \cdot \Sigma p},$$

where r = scoring scale range (in this study $r = 5$), Σp = number of comparable characters in the two individuals, $\Sigma |d|$ = sum of absolute values of differences in the characters of the two individuals. It is important to keep the range of the classification scale (r) the same for all characters.

The similarity values between all pairs of strains have been analysed not by the method of 'single linkage' (Sneath, 1957*b*), which partially distorts the complicated spatial relations expressed by the mutual similarity of strains (Lysenko & Sneath, 1959), but using another clustering method. In order to add a strain to a cluster (one strain or a group of strains), it must have a minimum number of links to the members of the cluster that are above the similarity value that is being examined. This minimum number is defined by the following scheme:

No. in cluster	Minimum links
1	1
2	1
3	2
4	2
5 and more	3

In this way the deformation of the actual spatial relations caused by their arrangement in a linear scale is decreased. Apart from this, another rule was made. In each step of clustering (1 % S) clusters were checked and when a strain in any cluster had a greater number of links to another cluster than to its own, it was transferred. In general, strains from smaller clusters were transferred to bigger ones. By means of these two rules about the addition and the transfer of strains the whole process has been done without using a computer.

RESULTS

The similarity values and the clustering are given in Tables 2 and 3. After some consideration the value $S = 85\%$ has been chosen as the 'species' level. Consequently, there are 11 groups (85-phenons according to the nomenclature of Sneath & Sokal, 1962) in the set.

While designating the groups I have primarily relied upon *Bergey's Manual* (1948, 1957) and tried to find for each 85-phenon the name of a species whose original description best corresponds with that phenon. The view of Evans, Bradford & Niven (1955) that *Micrococcus* and *Staphylococcus* constitute two different genera which can be with certainty differentiated on the basis of the 'fermentative' technique of glucose utilization, has been accepted. Validity of this point of view was clearly demonstrated by the quite different ratio of DNA bases in these genera (Silvestri & Hill, 1965).

A survey of characters of the phenons is shown in Table 4.

DISCUSSION

Classification techniques. The first clustering technique used in numerical taxonomy was 'single linkage' (Sneath, 1957*b*), later modified to the 'average linkage' method (Sokal & Michener, 1958) or that given by Hubálek (1964). A more objective determination of groups connected with a 'reasonable measure of inhomogeneity' has been suggested by Rogers & Tanimoto (1960). The use of factorial analysis has made possible a fully automatic and objective analysis of data (Hill *et al.* 1965; Gyllenberg, 1965; Poncet, 1967). Other methods introduced include 'probabilistic similarity

Table 2 (part 1). *Similarity values.*

S·D ³	80	105	144	149	209	248	265	291	296	335	357	351	357	407	440	443	523	531	536	546	555
80																					
105	867																				
144	833	861																			
149	861	886	827																		
209	828	870	830	875																	
248	832	895	882	836	863																
265	821	832	845	846	875	879															
291	833	791	821	823	788	798	809														
296	837	840	884	834	812	847	884	821													
335	814	856	861	850	846	877	857	770	882												
357	768	829	820	800	818	879	843	741	816	871											
351	826	865	870	846	847	851	868	775	877	846	836										
357	836	814	748	839	850	832	850	798	816	807	800	818									
407	788	795	764	798	830	802	829	768	825	798	777	798	873								
440	821	857	859	843	848	836	864	836	861	829	830										
443	788	812	807	818	858	823	843	730	856	861	825	874	829	848	886						
523	807	829	834	807	836	861	886	759	863	889	886	877	807	809	909	864					
531	786	818	813	814	850	813	864	750	830	871	879	868	832	841	868	871	896				
536	846	836	820	846	868	861	871	818	845	839	805	854	954	895	850	839	857				
546	872	835	840	814	814	825	839	833	875	811	754	840	800	805	843	837	811	796	818		
555	856	821	847	804	793	818	818	868	854	804	746	830	789	770	811	784	786	793	811	909	
617	868	854	863	841	875	833	852	797	835	809	770	835	813	786	834	814	809	795	834	854	851
631	800	849	840	807	853	842	868	735	847	867	864	872	796	798	882	884	893	871	825	839	804
683	813	848	818	873	841	849	805	850	870	860	850	838	851	875	854	882	871	871	829	811	
732	840	791	816	805	777	788	795	870	853	781	777	814	784	793	788	765	784	770	813	854	865
819	852	855	893	848	813	834	863	864	893	838	788	863	784	782	848	795	838	825	809	884	891
1024	819	791	839	784	746	791	788	821	863	819	738	804	759	757	813	793	795	759	770	979	840
1681	818	793	840	768	754	793	811	830	840	807	750	809	743	752	829	767	814	786	768	884	874
169	817	787	811	809	787	798	824	898	849	769	769	776	824	778	813	761	794	783	839	833	830
276	866	815	849	822	815	818	895	889	853	815	778	807	815	784	844	786	829	822	826	887	895
530	829	786	805	778	767	793	815	844	816	775	753	796	789	751	818	800	767	807	858	864	
550	813	780	782	776	762	776	813	833	807	758	755	791	787	749	798	758	776	762	802	851	840
656	805	777	782	773	745	780	773	864	771	738	752	759	791	739	766	734	755	745	802	813	823
1335	845	795	746	780	769	745	816	833	811	773	762	798	795	746	816	765	809	780	805	858	858
1423	820	791	793	780	784	788	820	818	821	777	780	788	777	754	834	784	816	780	791	852	830
1680	833	791	786	765	762	787	798	831	796	751	769	791	791	753	805	758	787	769	804	840	847
1134	863	827	832	848	795	827	813	829	839	813	773	791	845	796	802	795	780	770	841	823	827
163	856	832	837	861	853	839	836	840	816	804	796	823	825	791	814	798	786	796	843	842	825
166	819	809	814	789	798	805	820	853	793	749	763	758	821	779	791	758	759	763	841	812	823
183	865	847	853	855	840	805	827	832	863	840	784	818	820	811	827	818	802	788	839	833	840
240	835	832	823	821	800	825	821	847	842	807	793	812	793	752	804	774	779	771	814	828	821
266	842	779	745	782	779	797	796	854	802	768	761	777	804	770	764	739	761	750	829	809	846
247	877	816	804	813	820	830	813	843	800	770	763	766	827	800	795	769	777	773	848	838	823
852	816	798	809	799	805	788	802	807	783	763	763	765	766	764	798	765	763	748	795	816	812
249	814	789	816	804	782	811	782	873	813	761	761	782	825	791	796	766	757	757	839	829	839
308	833	819	779	841	823	805	802	744	842	875	780	846	795	807	820	849	827	830	816	840	823
294	740	698	725	693	674	705	693	826	735	688	721	714	693	684	689	682	704	682	719	765	786
688	798	713	761	738	691	730	718	839	757	713	736	736	719	709	718	686	729	704	753	788	827
810	736	697	733	702	689	721	705	835	730	693	702	702	720	704	695	677	695	677	748	774	802
824	748	698	711	691	677	705	688	814	725	680	717	702	695	700	691	680	705	680	727	759	777
642	758	744	767	773	696	740	707	813	753	722	715	740	707	691	693	671	693	678	733	760	796
848	722	711	724	700	645	678	649	733	709	660	685	704	649	647	649	627	653	627	678	720	756
310	756	725	735	739	695	715	702	795	720	689	687	727	698	674	683	669	670	659	709	747	791
644	780	757	763	769	739	732	725	804	725	718	698	739	716	700	731	704	691	709	738	770	796
46	765	712	744	693	684	698	686	774	728	688	654	714	686	677	693	668	671	671	721	761	768
52	789	707	723	706	696	699	695	805	738	686	655	709	695	685	695	663	662	662	724	763	791
135	730	677	689	675	628	656	643	784	672	639	625	665	661	655	639	630	629	596	675	740	751
253	767	746	737	738	696	718	720	751	733	693	666	716	691	671	709	688	680	688	705	739	760
257	779	761	739	757	712	734	729	760	739	719	696	735	704	680	739	700	700	714	765	772	
336	725	728	731	732	739	721	734	691	716	746	720	765	720	689	730	747	734	738	727	739	702
362	730	779	795	764	765	784	764	753	756	747	729	760	743	709	761	735	743	754	750	772	786
661	740	695	744	736	702	663	696	756	682	663	654	675	664	641	686	672	668	675	675	726	754

Table 2 cont. (part 2)

$S \cdot 10^3$	617	631	685	732	849	904	961	109	276	530	556	636	1335	1423	1680	174	163	166	183	240	266
80																					
105																					
144																					
149																					
209																					
248																					
265																					
291																					
296																					
335																					
357																					
391																					
357																					
407																					
440																					
443																					
523																					
531																					
536																					
546																					
555																					
617																					
631	816																				
683	811	884																			
732	793	788	844																		
819	861	830	811	893																	
1404	811	795	804	828	857																
1601	812	800	820	844	877	882															
169	785	769	819	837	837	785	809														
276	842	822	830	864	876	849	855	867													
530	813	778	795	838	858	824	851	891	902												
550	775	776	786	836	844	793	816	896	882	940											
656	754	752	800	829	867	782	813	904	860	882	902										
1335	822	795	807	826	847	829	845	874	900	936	913	876									
1423	825	816	829	829	839	818	834	874	864	893	858	836	902								
1680	778	780	793	822	829	789	813	889	870	922	931	902	935	876							
174	814	788	782	825	839	804	784	822	827	791	789	800	800	804	793						
163	819	804	805	837	841	777	779	843	829	815	798	798	805	820	791	855					
166	814	770	786	842	825	781	788	863	849	824	815	836	811	807	824	879	872				
183	853	802	821	814	843	807	802	811	809	780	764	786	782	791	778	889	865	839			
240	802	779	802	816	841	798	807	850	818	815	813	827	813	823	827	877	867	879	868		
266	805	744	795	830	823	763	765	861	826	800	804	823	805	809	795	870	858	847	834	846	
247	829	788	815	850	829	796	788	856	845	845	836	836	836	829	840	836	855	850	829	852	823
852	835	777	818	807	807	790	812	804	811	791	764	768	778	811	771	805	854	856	832	833	809
249	805	743	796	852	834	788	811	872	840	849	831	892	813	791	835	834	835	863	845	829	821
308	793	830	820	790	811	800	791	743	795	756	747	729	751	743	729	800	809	737	814	770	774
294	689	688	746	798	763	749	733	813	789	775	784	813	755	741	791	720	709	746	728	765	797
688	714	705	753	854	811	771	802	827	820	820	804	849	796	782	807	755	759	764	746	763	830
810	702	696	732	807	775	754	749	841	805	807	850	789	761	807	736	753	779	737	774	821	
804	696	680	727	793	750	732	723	819	776	795	793	821	778	761	807	736	759	764	739	784	809
642	712	689	720	833	811	753	773	794	791	781	786	833	767	760	789	749	769	771	731	773	784
848	698	656	684	746	767	727	744	735	724	736	727	771	746	713	738	691	745	720	695	718	717
310	704	678	700	802	756	711	710	800	757	764	785	804	732	719	774	765	780	789	753	769	844
644	771	714	731	780	776	738	761	764	776	756	737	776	754	755	741	742	813	755	779	770	818
46	721	670	705	795	738	707	733	769	742	753	755	752	725	741	744	748	790	791	739	775	786
52	748	682	705	813	751	713	739	778	763	760	758	755	733	751	744	773	800	816	755	775	825
135	668	646	666	770	709	728	730	769	738	727	718	759	711	700	704	709	705	735	696	716	783
253	744	693	682	751	754	719	718	707	748	722	691	704	720	736	713	757	774	753	751	746	720
257	742	716	716	770	763	742	730	718	767	753	718	720	744	766	729	766	793	742	760	775	841
336	765	721	741	691	748	733	741	689	741	700	669	671	695	736	655	724	788	702	746	718	725
362	795	730	752	746	773	767	765	735	764	738	711	716	729	766	707	734	775	735	756	723	747
661	763	649	677	721	741	725	742	726	769	746	704	713	736	730	704	680	754	714	744	742	744

Table 2 *cont.* (part 3)

$S \cdot 10^3$	247	852	249	308	294	688	810	824	642	848	310	644	46	52	155	253	257	336	362	661
80																				
105																				
144																				
149																				
209																				
248																				
265																				
291																				
296																				
335																				
337																				
351																				
357																				
407																				
410																				
443																				
523																				
531																				
536																				
546																				
555																				
617																				
631																				
683																				
732																				
819																				
1424																				
1681																				
169																				
276																				
530																				
550																				
656																				
1335																				
1423																				
1680																				
134																				
163																				
166																				
183																				
210																				
266																				
247																				
652	864																			
249	845	802																		
308	764	740	741																	
294	759	704	800	686																
688	793	739	813	729	873															
810	796	730	800	688	896	879														
824	764	707	788	686	948	869	889													
642	767	727	809	707	787	867	844	782												
848	716	702	744	664	835	859	826	812	880											
310	759	733	774	702	805	844	838	793	826	800										
644	780	759	747	741	754	831	802	762	802	780	817									
46	759	763	746	714	698	766	742	713	787	740	784	757								
52	780	777	771	718	732	802	770	747	743	755	831	780	943							
135	702	711	754	693	775	791	809	755	747	736	805	759	761	800						
253	739	747	702	709	707	736	719	696	706	684	778	791	732	773	739					
257	759	760	714	739	719	766	746	716	718	689	816	811	751	786	768	935				
336	681	712	666	747	623	655	632	636	684	622	669	732	672	663	672	723	742			
362	720	753	775	704	670	693	675	652	696	675	718	768	684	686	677	760	761	840		
661	709	718	700	707	721	720	719	713	711	700	736	793	663	696	737	773	783	788	807	

Table 3. *Clustering of micro-organisms*

Number of clusters in the set is given in parentheses.

$S \geq 0.990$:	— (62)
0.980:	— (62)
0.970:	— (62)
0.960:	— (62)
0.950:	A. 357, 536 (61)
0.940:	A. ditto; B. 46, 52; C. 294, 824; D. 530, 550 (58)
0.930:	A., B., C. ditto; D. ditto + 1335, 1680; E. 253, 257 (55)
0.920:	ditto (55)
0.920:	ditto (55)
0.900:	A., B., C. ditto; D. ditto + 276; E. ditto; F. 169, 656; G. 410, 523; H. 546, 555 (51)
0.890:	A. ditto + 407; B. ditto; C. ditto + 810; D., E. ditto; F. ditto + 291; G. ditto + 531; H. ditto; I. 105, 248; J. 144, 296, 819; K. 335, 351 (43)
0.880:	A., B., C. ditto; D. ditto + 169, 656; E. ditto; F. 291; G. ditto + 443, 631; H. ditto + 1681; I. ditto + 149; J., K. ditto; L. 134, 183; M. 642, 848 (37)
0.870:	A., B. ditto; C. ditto + 688; D. ditto + 1423; E. ditto; F. 291, 249, 732; G. ditto + 683, 335, 337, 351; H. ditto + 819, 1424; I. ditto + 209, 265; J. 144, 296; K. —; L. ditto + 166, 210; M. ditto; N. 163, 617; ·80, 247 (23)
0.860:	A. 357, 407; B., C. ditto; D. ditto + 291, 555, 732; E. ditto; F. 249; G. ditto + 105, 144, 209, 248, 265, 296, 536, 819; H. 546, 1424, 1681; I. 149; J., K. —; L. ditto + 80, 163; M. ditto; N. 617; ·247, 852 (21)
0.850:	A. —; B., C. ditto; D. —; E. ditto; F. —; G. ditto + 276, 555, 546, 732, 1424, 1681; 291, 530, 169, 550, 1335, 1680, 656, 1423, 357, 407, 617, 80, 183, 163, 134, 166, 210, 149, 249, 247, 852, 266; H., I., J., K., L. —; M. ditto; N., O. —; P. 336, 362 (11)
0.840:	B. ditto; C. ditto + 642; E. ditto; G. ditto + 308; M. 848; P. ditto. A., D., F., H., I., J., K., L., N., O. — (10)
0.830:	B., E. ditto; G. ditto + 294, 310, 642, 688, 810, 824, 848; P. ditto. The remaining — (7)
0.820:	ditto (7)
0.810:	B. 46; E. 253; G. ditto + 52, 257, 644; P. ditto (6)
0.800:	B. 46; E. 253; G. ditto + 135; P. 336, 362, 661 (4)
0.790:	B. —; E. 253; G. ditto + 46; P. ditto (3)
0.780:	E. —; G. ditto + 253, 336, 362, 661; P. — (1)

'ditto': The same strains as the previous step.

index' (Goodall, 1966) for direct significance testing of classification relationships; *t*-test for a statistical comparison of more or less similar groups of strains (Hubálek, 1964; Tsukamura, 1967); calculation of taxonomic relations derived from the theory of entropy (Hall, 1967); 'furthest neighbour sorting' and 'flexible sorting' (Lance & Williams, 1967). At present it is difficult to judge which of these methods is most advantageous. A comparison of some of them has been made for example by Hill *et al.* (1965) and 'tMannetje (1967), but without any definite conclusion. However, the results obtained by various grouping techniques may be different to a certain extent. Up to now, the 'principal component analysis' and 'gradient method' (Hill *et al.* 1965) seem to be very objective methods in taxometrics.

Taxonomic problems. The view that 85-phenons in this study would be about at the level of species seems to be supported by the results of Rosypal *et al.* (1966). They have studied, among others 11 strains also common to the set in this investigation; the strains fell on DNA base composition into three different groups:

Strain CCM no.	85-phenon	Rosypal <i>et al.</i> (1966) <i>Micrococcus</i> group
144, 210, 247, 248, 265, 266, 523, 852, 1335	I	1: 70.8 to 73.3 moles % GC
810	III	3: 65.8 to 67.0 moles % GC
310	V	2: 67.5 to 69.5 moles % GC

Table 4. *Scoring of characters in 85-phenons*

85-phenons												
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI
		Strains CCM no.										
		45 strains (see Table 1)	308	294, 688 810, 824	642, 848	310	644	46, 52	135	253, 257	336, 362	66
Characters no.	Mean	Scores and range										
1	3.9	3-5	4	2.5-4	4	3	4	4	3	5	4-5	3.5
2	2.5	(0) 1-4.5	5	0-1	0.5-1	3	2	1	0	3-4	3-4	0.5
3	2.7	(0) 1-5	4	0.5-1.5	1-2	2.5	3	1	1	3.5-4	3-4.5	2.5
4	0.1	0 (1.5)	0	0-3	0-4	0	0	0	0	0	0	0
5	1.0	0-4	0	0	0	3.5	1.5	3.5	0.5	4.5-5	0	1
6	1.8	0-3.5 (4)	1	2-3	3-4	1.5	2	3	2.5	2-3	3	3
7	1.7	0-4 (5)	4	0	0	0	1	0	0	0.5-1	1-4	0
8	1.4	0-3 (5)	2.5	0-1	0	0	2	2-3	0	2.5-3.5	3	5
9	2.8	(1) 2-4	3	2-3	2	2	2	3	2	2-3	3	2
10	2.3	0-5	4.5	0-1.5	0.5-1	1	2.5	2-3	1	1.5-3	3-4.5	1
11	0.2	0-1 (2)	0	0.5-3	3-5	2	2.5	3-4	1.5	0	0-1	0
12	2.0	(0.5) 1-3 (4)	3	1	1	1	1	1	1	2	2	1
13	3.8	(1) 3-5	4	2-4	3-4	—	3	1	4	1	3-5	3
14	4.4	(2) 3-5	5	4.5-5	4	—	3.5	1-2	5	3	3-5	4
15	0.3	0-1 (2)	0	0	0-1.5	4	0	0-2	1	0	0-1	0
16	0.2	0-1 (2)	0	0	0	0	0	0	0	0	0	0
17	0.2	0 (5)	0	0	0	0	0	0	0	0	0	0
18	4.1	(2) 3-5	3	2-2.5	2.5-3	2.5	1	4-5	2	3.5-5	2-4	3.5
19	3.9	2.5-5	5	2-3	2-3	3	1	3-3.5	4	3-4	2-4	3
20	3.0	2-4	3	2-3	2	3.5	—	2-2.5	2.5	3	3.5-4	3
21	0.6	0-2 (4)	2	0	0-2.5	0	0	0	5	1	0	0
22	0.6	0-3	3.5	0	0-1.5	0	0	0	0	0	0	0
23	0.4	0-2 (2.5)	0	0	0	0	0	0-1	0.5	3	4-4.5	4.5
24	0.1	0-0.5 (1)	0	0	0	0	0	0	3	3.5	4-4.5	4.5
25	0.0	0	0	0	0	0	0	0	1	0	3	0.5
26	1.1	0-2	1	0-2	0-2	0	2	0	0	1-2	2	2.5
27	3.4	2-4 (4.5)	4	2-3	2.5	2	2.5	1-2	1.5	2-2.5	3	4
28	3.2	(1.5) 2-4 (4.5)	4	2-3	2	2	2	1.5-2	1	2.5-3	1-2.5	3
29	1.6	0.5-2	0.5	1.5-4	1-2	2	3	0.5-1	0	3.5-4	0.5-2	2
30	1.2	(0) 0.5-2	1	1-4.5	4.5	1	3	0-0.5	1.5	2.5-4.5	1-1.5	2
31	1.7	(0) 0.5-2 (4)	1	2-4	2-4	3	0.5	1.5	4	2.5	0-3	4
32	1.7	0-2 (3)	3	0-2	2	0.5	2	1.5-2	0	0.5-1	2-2.5	4
33	0.4	0-1 (2)	1	0-0.5	2.5	2	0	0	0	1	5	5
34	1.8	0-5	1	0-2	5	3	5	0	0	2	4-5	5
35	2.2	0-4 (4.5)	1.5	0	0	0	4	0	0	3	2.5-4	5
36	3.3	1.5-4 (5)	5	0-2.5	0-1	0	4	0	4	4	3.5-4	5
37	1.3	0-2.5	2	0	0	0	0	0	0	2	2	2
38	3.4	(0) 2-5	1	0	2.5-3.5	0	0	1.5-4	0	0	4	0
39	3.0	(0) 1.5-4 (4.5)	3	0	0	0	0	0	0	0	0	0
40	3.0	(0) 2-4	2.5	0	—	0	0	5	0	0	3-4	0
41	2.9	(0) 2-4	3	0	—	0	2	2-3	0	0	1-2	0
42	2.6	(0) 1-4 (5)	4	0-4	0	0	0	3-3.5	2	0-0.5	2-4	4
43	0.8	0-2.5	2	0-0.5	0-0.5	0	0.5	0	0	0	0-2	1
44	0.7	0-2 (2.5)	0	0-0.5	0-0.5	0	0	0	0	1	0-0.5	1
45	0.9	0-3	0	0	0-1	0	0	0	0	0	0	0
46	2.4	0-5	0	0-2	0	0.5	2	0	0	0	2.5-3.5	3
47	0.3	0-2 (2.5)	5	0	3-4	3	5	5	5	3	0.5-2.5	5
48	0.0	0 (1)	0	0	0	0	0	5	4.5	3-4	0	0
49	2.7	(1.5) 2-3 (5)	2	1.5-3	2.5	3	2.5	3	5	2.5-3	2.5-5	3
50	4.0	(2.5) 3-5	4	3.5-5	2.5	4	4	3	4	4	4-5	5
51	3.1	(1) 2-4 (5)	3	2	2	4	3	3	4	3	5	4
52	3.7	3-5	4	4-5	4	3	4	3	5	3	3-4	4
53	1.6	0-3	2	3	3	3	3	2	3	2-3	2-4	3
54	3.4	(2) 3-4 (5)	3	0-3	0-3	3	4	3	4	3-4	5	4
55	2.6	1-4	2	0	1-2	2	3	3	3	3	4	3
56	3.4	0-5	1.5	3-4.5	4-5	0	0	0	0	0	0	0
57	0.8	0-5	0	0-1	0	0	0	0	0	0	0	0

Legend: number in parentheses: the score is achieved by 1 or 2 strains only; —, could not be scored.

Determination of phenons in accordance with *Bergey's Manual* (1952; 1948):

85-phenon I: Approximately as *Micrococcus flavus*; 85-phenon II: *M. citreus*; 85-phenon III: *M. luteus*; 85-phenon V: *M. aurantiacus*; 85-phenons IV, VI, VII: *Micrococcus* spp.; 85-phenon IX: *Staphylococcus epidermidis*; 85-phenon X: *S. conglomeratus*; 85-phenon XI: *S. varians*; 85-phenon VIII: *Staphylococcus*

85-phenon I (*Micrococcus flavus*) includes a number of strains with cells occurring in the form of packets and which are usually placed into the genus *Sarcina*. The whole phenon I may be divided in three morphological types:

1. 'Sarcinas'—strains with cells arranged in typical packets of cocci: 19 strains (CCM 134, 149, 163, 209, 248, 265, 296, 335, 337, 351, 357, 410, 443, 523, 531, 536, 631, 683, 819).

2. 'Micrococci'—strains with cells arranged in more or less typical clusters of cocci: 17 strains (CCM 80, 169, 247, 276, 291, 530, 546, 550, 555, 656, 732, 852, 1335, 1423, 1424, 1680, 1681).

3. Strains with 'intermediary' arrangement of cocci: 9 strains (CCM 105, 144, 166, 183, 210, 249, 266, 407, 617).

The formation of packets is not a quite constant feature in aerobic sarcinas (Kocur & Martinec, 1962; Hubálek, 1964) and correlates with a number of other morphological properties (turbidity in broth is weak or none, there are conglomerates in liquid medium, sediment is grainy, colonies matt to grainy) but not with physiological or biochemical properties (only the activity of proteolytic enzymes in sarcinas, as well as the production of hydrogen sulphide, appears to be somewhat higher; it is interesting to note that all the sarcinas studied, without exception, were proteolytic). In order to compare both basic morphological types in the 85-phenon I, six strains of the morphological type 1 and six strains of the type 2 were randomly selected and the similarity was calculated between all these strains, after the characters nos. 2, 3, 6, 7, 10 and 12 (which are directly influenced by the morphological type) had been eliminated. Then the mean intragroup similarity values were found for both types and also the mean value of intergroup similarity ($\bar{x}_{1.2}$), and their standard deviations:

$$\begin{aligned}\bar{x}_1 &= 86.487\% S, & s_1 &= \pm 3.895\% S, \\ \bar{x}_2 &= 84.527\% S, & s_2 &= \pm 3.406\% S, \\ \bar{x}_{1.2} &= 82.939\% S, & s_{1.2} &= \pm 1.897\% S.\end{aligned}$$

While comparing these S -mean values by t -test, the Cochran modification of the Behrens-Fisher method (see Snedecor, 1957) has been used because

$$n_1 \neq n_2 \quad (n_1 = 15, n_2 = 36) \quad \text{and} \quad \sigma_1 \neq \sigma_2 \quad (F_{1/1.2} = 4.22^{**}, F_{2/1.2} = 3.22^{**}).$$

The formula is written as follows:

$$t' = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\left(s^2_{\bar{x}_1} + s^2_{\bar{x}_2}\right)}},$$

and the corresponding values are

$$t'_{1/1.2} = 3.366, \quad t'_{2/1.2} = 1.699.$$

Distribution of t' -values is different from the Student's t ; the t' -tabular values have been estimated by the formula:

$$t'_{0.05} = \frac{s^2_{\bar{x}_1} \cdot t_{0.05_1} + s^2_{\bar{x}_2} \cdot t_{0.05_2}}{s^2_{\bar{x}_1} + s^2_{\bar{x}_2}} = 2.13$$

$$\text{for } t'_{0.05_{1/1.2}} \text{ and } t'_{0.05_{2/1.2}}, \text{ too.}$$

According to Tsukamura (1967), two samples of more or less taxonomically similar strains may be assumed to be two distinct subspecies if *both* intragroup similarity

mean values are different from the intergroup one; the difference is estimated by *t*-test for $P = 0.05$. In our case, the value $t'_{2/1.2}$ indicates that a classification of both samples (randomly drawn from the morphological types 1 and 2) as two different subspecies is not justified and obviously they cannot be considered as separate species or genera.

Discussions on the unsuitability of placing aerobic sarcinas into a separate genus may be found in the literature (Winslow & Winslow, 1908; Shaw *et al.* 1951; Evans *et al.* 1955) but only Kocur & Martinec (1962) transferred them into the genus *Micrococcus*, and the present communication has confirmed this view. It is probable (Hubálek, 1964) that aerobic 'sarcinas' are R-dissociated forms of proteolytic micrococci.

The genus *Sarcina*, however, should be kept for the anaerobic sarcinas: *S. ventriculi*, *S. maxima*, *S. methanica*, *S. barkeri*.

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REFERENCES

- ABD-EL-MALEK, Y. & GIBSON, T. (1948). Studies in the bacteriology of milk. II. The staphylococci and micrococci of milk. *J. Dairy Res.* **15**, 249.
- BAIRD-PARKER, A. C. (1963). A classification of micrococci and staphylococci based on physiological and biochemical tests. *J. gen. Microbiol.* **30**, 409.
- BAIRD-PARKER, A. C. (1965). The classification of staphylococci and micrococci from world-wide sources. *J. gen. Microbiol.* **38**, 363.
- Bergey's Manual of Determinative Bacteriology* (1948) 6th ed. Ed. by R. S. Breed, E. G. D. Murray and A. P. Hitchens. Baltimore: The Williams and Wilkins Co.
- Bergey's Manual of Determinative Bacteriology*. (1957). 7th ed. Ed. by R. S. Breed, E. G. D. Murray and N. R. Smith. Baltimore: The Williams and Wilkins Co.
- BREED, R. S. (1952). The type species of the genus *Micrococcus*. *Int. Bull. bact. Nomencl. Taxon.* **2**, 85.
- EVANS, J. B., BRADFORD, N. L. & NIVEN, C. F. (1955). Comments concerning the taxonomy of the genera *Micrococcus* and *Staphylococcus*. *Int. Bull. bact. Nomencl. Taxon.* **5**, 61.
- GOODALL, D. W. (1966). A new similarity index based on probability. *Biometrics* **22**, 882.
- GYLLENBERG, H. G. (1965). A model for computer identification of micro-organisms. *J. gen. Microbiol.* **39**, 401.
- HALL, A. V. (1967). Methods for demonstrating resemblance in taxonomy and ecology. *Nature, Lond.* **214**, 830.
- HILL, L. R. (1959). The Adansonian classification of the staphylococci. *J. gen. Microbiol.* **20**, 277.
- HILL, L. R., SILVESTRI, L. G., IHM, P., FARCHI, G. & LANCIANI, P. (1965). Automatic classification of staphylococci by principal-component analysis and a gradient method. *J. Bact.* **89**, 1393.
- HUBÁLEK, Z. (1964). *Problém Sarcina*. Thesis: J. E. Purkyně University, Brno.
- KOCUR, M. & MARTINEC, T. (1962). Taxonomická studie rodu *Micrococcus*. *Folia Pšir. fak. UJEP* **III**, 3.
- LANCE, G. N. & WILLIAMS, W. T. (1967). A general theory of classificatory sorting strategies. *Comput. J.* **9**, 373.
- LYSENKO, O. & SNEATH, P. H. A. (1959). The use of models in bacterial classification. *J. gen. Microbiol.* **20**, 284.
- POHJA, M. S. (1960). Micrococci in fermented meat products. Classification and description of 171 different strains. *Acta agralia fenn.* **96**, 1.
- POHJA, M. S. & GYLLENBERG, H. G. (1962). Numerical taxonomy of micrococci of fermented meat origin. *J. appl. Bact.* **25**, 341.

- PONCET, S. (1967). A numerical classification of yeasts of the genus *Pichia* Hansen by a factor analysis method. *Antonie van Leeuwenhoek* **33**, 345.
- ROGERS, D. J. & TANIMOTO, T. T. (1960). A computer program for classifying plants. *Science, N.Y.* **132**, 1115.
- ROSYPAL, S., ROSYPALOVÁ, A. & HOŘEJŠ, J. (1966). The classification of micrococci and staphylococci based on their DNA base composition and Adansonian analysis. *J. gen. Microbiol.* **44**, 281.
- SHAW, C., STITT, J. M. & COWAN, S. T. (1951). Staphylococci and their classification. *J. gen. Microbiol.* **5**, 1010.
- SILVESTRI, L. G. & HILL, L. R. (1965). Agreement between deoxyribonucleic acid base composition and taxometric classification of Gram-positive cocci. *J. Bact.* **90**, 136.
- SNEATH, P. H. A. (1957*a*). Some thoughts on bacterial classification. *J. gen. Microbiol.* **17**, 184.
- SNEATH, P. H. A. (1957*b*). The application of computers to taxonomy. *J. gen. Microbiol.* **17**, 201.
- SNEATH, P. H. A. & SOKAL, R. R. (1962). Numerical taxonomy. *Nature, Lond.* **193**, 855.
- SNEDECOR, G. W. (1957). *Statistical Methods Applied to Experiments in Agriculture and Biology*. 5th ed., Ames: The Iowa State College Press.
- SOKAL, T. R. & MICHENER, C. D. (1958). A statistical method for evaluating systematic relationships. *Kans. Univ. Sci. Bull.* **38**, II, 1409.
- *TMANNETJE, L. (1967). A re-examination of the taxonomy of the genus *Rhizobium* and related genera using numerical analysis. *Antonie van Leeuwenhoek* **33**, 477.
- TSUKAMURA, M. (1967). A statistical approach to the definition of bacterial species. *Jap. J. Microbiol.* **11**, 213.
- WINSLOW, C.-E. A. & WINSLOW, A. R. (1908). *The Systematic Relationships of the Coccaceae with a Discussion of the Principles of Bacterial Classification*. New York: J. Willey & Sons.