The Classification of Micrococci and Staphylococci Based on their DNA Base Composition and Adansonian Analysis

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SUMMARY

Species of the genus *Staphylococcus* have a guanine + cytosine (GC) content in DNA within the range 30.7–36.4 moles %. All the Gram-positive cocci so far designated as micrococci and sarcinas which produce acid from glucose when grown under anaerobic or aerobic conditions and have a GC content in DNA within this range should be also classified as *Staphylococcus*. On the other hand species of the genus *Micrococcus* have a GC content in DNA within the range 66.3–73.3%. All the Gram-positive cocci hitherto designated as sarcinas and staphylococci which do not produce acid from glucose under aerobic or anaerobic conditions or only aerobically and which have a GC content in DNA within this range, should be also classified as *Micrococcus*. The range of GC content as outlined is approximate; it will be made more accurate (perhaps a little broader) when more data are available on the DNA base composition of the species belonging to the family Micrococcaceae.

On the basis of their DNA base composition the strains belonging to the genus *Micrococcus* are divided into three groups: group 1 (70.8–73.3 moles % GC); group 2 (67.5–69.5 moles % GC); group 3 (66.3–67.0 moles % GC). Similarly the strains belonging to the genus *Staphylococcus* have been divided into three groups: group 5 (36.4 moles % GC); group 6 (33.3–34.2 moles % GC); group 7 (30.7–32.7 moles % GC). Strains within each group are believed to be phylogenetically related. When subjected to Adansonian analysis, the strains of the individual groups were clustered into subgroups. Each subgroup comprised the strains with approximately the same DNA base composition and a high % similarity of physiological and biochemical characters. Strains clustered into subgroups are believed to be genetically closely related, some may be identical. The following subgroups are suggested: micrococcus subgroups 1a, 1b, 2a, 2b, 2c, 3a, 3b; staphylococcus subgroups 5a, 6a, 7a. Arabic numerals refer to the designation of the group.

The classification of micrococci presented in this paper is substantially the same as that of Baird-Parker (1965). The authors are of the same opinion as Baird-Parker (1965) that *Micrococcus denitrificans* should be reclassified with the Gram-negative genera.

INTRODUCTION

The study of phylogenetic relationships among bacterial species made on the basis of their DNA base composition leads to conclusive results only when it concerns species of the same morphology, i.e. usually the species belonging to the

same family. Even when two morphologically different bacteria belonging to different families have the same DNA base composition, it cannot be concluded that they are phylogenetically related. Their morphological difference suggests that they are phylogenetically distant. In this case similarity in DNA base composition does not express the fact that their DNA has a similar sequence of bases. On the other hand, organisms with the same morphology and DNA base composition are usually phylogenetically related. However, their phylogenetic relationship is of different degree and they may, therefore, differ from one another more or less physiologically and biochemically. Only the organisms that are known to have the same DNA base composition and high degree of similarity in physiological and biochemical characters may be considered to be closely related genetically. It is, therefore, believed that before separating a set of organisms into species it would be better to classify them into groups, each comprising the strains not differing in DNA base composition and having a high degree of similarity of physiological and biochemical characters. On the basis of these considerations we have attempted to classify bacteria originally included in the genera *Micrococcus*, *Staphylococcus* and *Sarcina*. The DNA base composition in these cocci has been recently determined by Rosypalová, Boháček & Rosypal (1966*a, b*). Rosypal, Boháček & Rosypalová (1966) and Rosypal & Rosypalová (1966). The strains with the same DNA base composition have been grouped together and then subjected to Adansonian analysis according to Sneath (1957) in order to cluster the strains which are similar not only as to DNA base composition, but also biochemically and physiologically. Such strains are believed to be closely related genetically.

METHODS

The strains studied were obtained from the Czechoslovak Collection of Micro-organisms (CCM) and are listed in Table 1 under the name received by the CCM from other collections and under the new name assigned to them in the CCM. The base composition of DNA preparations from these strains were determined from their denaturation temperature (T_m) by Rosypalová, Boháček & Rosypal (1966*a, b*), Rosypal *et al.* (1966), Rosypal & Rosypalová (1966).

Each bacterial strain was tested for physiological and biochemical characters. For each strain, the following 67 properties were used in the computer analysis: grouping of organisms (packets, clumps); colonies on glucose yeast-extract agar (rough, smooth); pigment production on glucose yeast-extract agar (yellow, violet, pink pigments); dissociation in pigmentation; hydrolysis of starch, casein and gelatin; reduction of nitrates and nitrites; production of catalase, urease and lipase; sensitivity to chloramphenicol, chlortetracycline, terramycin, tyrothricin, nystatin, bacitracin and lysozyme; hydrolysis of aesculin and salicin; methyl red test; effects on milk; Gram-reaction; growth on Simmon's citrate agar; production of acid from carbohydrates (glucose, sucrose, galactose, fructose, mannose, lactose, xylose, glycerol, adonitol, sorbitol, mannitol); oxidation of carbohydrates in phosphate buffer at pH 7 (acetate, lactate, glycerol, mannitol, dulcitol, sorbitol, arabinose, xylose, rhamnose, glucose, galactose, fructose, sucrose, maltose, lactose, starch); oxidation of amino acids in phosphate buffer at pH 7 (glycine, alanine, threonine, leucine, lysine, histidine, arginine, proline, tryptophan, asparagine, glutamic acid).

Data concerning these tests and the methods used are given in papers by Kocur & Martinec (1962), Rosypal, Kocur & Hořák (1963), Rosypal & Kocur (1963), Rosypalová & Rosypal (1966).

The results of each test were scored as positive (+) or negative (−). Similarity (*S*) values for pairs of strains were calculated according to the formula (Sneath, 1957):

Table 1. *List of strains studied*

Number in Table 2 and Fig. 2	Number of the strain and the name under which it was received by the CCM	Number of strain and its new name given by the CCM
1	<i>Staphylococcus flavocyaneus</i> NCTC, 7011	<i>Micrococcus luteus</i> 247
2	<i>Micrococcus</i> sp. L.A. 9.1*	<i>M. luteus</i> 852
3	<i>M. flavocyaneus</i> *	<i>M. luteus</i> 622
4	<i>M. flavocyaneus</i> L.A. 8.2*	<i>M. luteus</i> 851
5	<i>M. flavocyaneus</i> L.A. 8.1*	<i>M. luteus</i> 853
6	<i>M. flavus</i> ATCC, 400	<i>M. luteus</i> 210
7	<i>M. sodonensis</i> ATCC, 11880	<i>M. luteus</i> 144
8	<i>Sarcina subflava</i> ATCC, 381	<i>M. luteus</i> 559
9	<i>S. marginata</i> IFO, 3066	<i>M. luteus</i> 265
10	<i>S. flava</i> isolated by Kocur	<i>M. luteus</i> 309
11	<i>S. variabilis</i> IFO, 3067	<i>M. luteus</i> 266
12	<i>S. citrea</i> †	<i>M. luteus</i> 248
13	<i>S. pelagia</i> SIO	<i>M. luteus</i> 331
14	<i>Micrococcus luteus</i> ATCC, 398	<i>M. luteus</i> 810
15	<i>Staphylococcus afermentans</i> NCTC, 2665	<i>M. luteus</i> 855
16	<i>Sarcina lutea</i> isolated by Kocur	<i>M. luteus</i> 523
17	<i>S. lutea</i> isolated by Kocur	<i>M. luteus</i> 310
18	<i>Micrococcus lysodeikticus</i> 53–20‡	<i>M. luteus</i> 1335
19	<i>Sarcina aurantiaca</i> ATCC, 146	<i>M. luteus</i> 686
20	<i>Micrococcus cyaneus</i> L.A. 1.1*	<i>M. luteus</i> 856
21	<i>Staphylococcus roseus</i> NCTC, 7520	<i>Micrococcus roseus</i> 146
22	<i>Micrococcus roseus</i> §	<i>M. roseus</i> 905
23	<i>M. rubens</i> ATCC, 412	<i>M. roseus</i> 633
24	<i>Sarcina erythromyxa</i> ATCC, 187	<i>M. roseus</i> 706
25	<i>S. ventriculi</i> CRIPP	<i>M. conglomeratus</i> 208
26	<i>Micrococcus denitrificans</i> NCIB, 8944	<i>M. denitrificans</i> 982
27	<i>M. conglomeratus</i> ATCC, 401	<i>M. conglomeratus</i> 547
28	<i>M. candidus</i> NCIB, 8610	<i>M. varians</i> 1044
29	<i>M. citreus</i> NCIB, 8611	<i>M. varians</i> 1046
30	<i>Staphylococcus lactis</i> NCTC, 7564	<i>M. varians</i> 884
31	<i>Sarcina oliva</i> †	<i>M. varians</i> 250
32	<i>Micrococcus infimus</i> SIO	<i>M. varians</i> 313
33	<i>M. euryhalis</i> SIO	<i>M. varians</i> 315
34	<i>M. varians</i> M 3	<i>M. varians</i> 529
35	<i>M. cerolyticus</i> ATCC, 12559	<i>M. varians</i> 901
	<i>Staphylococcus aureus</i> NCTC, 8511	
	<i>S. aureus</i> lysogenic derivative of NCTC, 8511	

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IH, Institute of Hygiene, Warsaw, Poland; NCIB, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen, Scotland; ATCC, American Type Culture Collection, Rockville, Md., U.S.A.; NCTC, National Collection of Type Cultures, London, England; CRIPP, Central Research Institute of Plant Production, Prague-Ruzyně, ČSSR; SIO, Scripps Institution of Oceanography, La Jolla, California, U.S.A.; IFO, Institute for Fermentation, Osaka, Japan; CCM, Czechoslovak Collection of Microorganisms, J. E. Purkyně University, Brno, ČSSR.

$S = n_s/n_s + n_d$ where n_s is the number of features positive for both organisms and n_d is the number of features positive for one but not the other organism. The Mean Similarity expressed as 'Triangle Mean' ΔS was also calculated (see Sneath, 1957). The MINSK electronic digital computer was employed. Copies of the program may be obtained from the authors.

RESULTS

Taking into consideration that the values of moles % GC content in DNA determined in individual strains from the denaturation temperature T_m , are reproducible within the limits of $\pm 1\%$, the whole set of strains can be divided into seven groups (see Fig. 1). In the sequels moles % GC means moles % guanine + cytosine in the given DNA preparation. Differences in moles % GC values within these groups are not considered to be significant. The borderlines between the respective groups are only approximate and may be modified as soon as new data on DNA base composition in micrococci and staphylococci are available. However, no substantial changes are to be expected. The following classification into groups is suggested:

Group 1, strains with a GC content within the range 70.8–73.3 moles %.

Group 2, strains with a GC content within the range 67.5–69.5 moles %.

Group 3, strains with a GC content within the range 65.8–67.0 moles %. As stated in the Discussion, the species *Micrococcus denitrificans* is not included in this group. The range of GC is therefore 66.8–67.0 moles %.

Group 4 is for the time being represented by only one strain with a GC content 54.2 moles % GC.

Group 5 is also represented by one strain with a GC content 36.4 moles % GC.

Group 6, strains with a GC content within the range 33.8–34.2 moles %.

Group 7, strains with a GC content within the range 30.7–31.1 moles %. This range is broader (30.7–32.7 moles % GC) when we take into account the data by Silvestri & Hill (1965).

When subjected to Adansonian analysis, the strains of individual groups were clustered into subgroups. Each subgroup comprised the strains with approximately the same DNA base composition and a high % of similarities in physiological and biochemical characters (see Table 2, Fig. 2). The intra-group and inter-group similarity values (ΔS or Triangle Mean) are given in Table 3. The success of the clustering is indicated by the intra-group S values. The subgroups were obtained at approximately 70 % ΔS (see Table 3).

Group 1 is divided into subgroups 1a ($\Delta S = 72\%$) and 1b ($\Delta S = 73\%$). The inter-group S value between these subgroups equals 59 % S .

Group 2 is formed of subgroups 2a ($\Delta S = 67\%$), 2b and 2c; subgroups 2b and 2c are represented by only one strain each. Subgroup 2b is very similar to subgroups 1a and 1b, though having a different moles % GC content.

Group 3 is formed of subgroups 3a, 3b and 3c, each represented by one strain. Subgroups 3a and 3b have great similarity to subgroup 1a, although differing from it by their DNA GC contents.

Groups 4, 5 and 6 have each only one subgroup: 4a, 5a and 6a ($\Delta S = 71\%$) and are quite dissimilar from all the above-mentioned groups and subgroups. There is a high percentage of similarity between subgroups 4a and 5a (inter-group S

Fig. 1. Diagrammatic representation of the differences in DNA base compositions between the strains studied. The strains not differing each from another by more than 2 % GC are believed to have a similar DNA base composition. They form, therefore, a group of phylogenetically related organisms.

Fig. 2. Diagrammatic representation of the S-value table, prepared by shading the squares according to S-values between the strains.

Table 2. The full table of *S* values (%) for micrococci, staphylococci and sarcinas after re-arranging the strains by the sorting procedure. The strains with similar GC contents in DNA were first grouped together and the members of each group were then sorted on the basis of their *S* values

Name of strain as received	Groups										Subgroups										Strain number																							
	1a										1b										2a																							
	3	4	5	1	7	8	9	6	11	13	16	10	12	15	18	21	23	24	22	28	29	30	25	27	17	19	20	3a	3b	3c	4a	5a	6a											
O in DNA within the range 70.8-73.3 moles %																																												
<i>Micrococcus flavoviridescens</i>	3	100																																										
<i>M. flavoviridescens</i>	4	89	100																																									
<i>Micrococcus</i> sp.	2	88	89	100																																								
<i>M. flavoviridescens</i>	5	79	89	100																																								
<i>Staphylococcus flavoviridescens</i>	1	84	85	79	85	100																																						
<i>Micrococcus sodonensis</i>	7	74	74	73	70	75	100																																					
<i>Sarcina subflava</i>	8	71	76	70	76	82	81	100																																				
<i>S. marginata</i>	9	71	72	70	68	73	81	83	100																																			
<i>Micrococcus flavus</i>	6	68	69	68	73	82	86	84	84	100																																		
<i>Sarcina variabilis</i>	11	67	72	70	76	77	71	78	73	79	100																																	
<i>S. pelagia</i>	13	65	66	74	75	71	69	76	71	78	88	100																																
<i>S. lutea</i>	16	60	69	63	69	70	88	79	70	76	74	68	100																															
<i>S. flava</i>	10	68	68	63	68	78	68	74	69	79	83	72	84	100																														
<i>S. citrea</i>	12	65	62	64	70	71	69	76	67	73	76	81	68	77	100																													
<i>Staphylococcus aerfermentans</i>	15	73	64	67	59	65	73	75	67	65	73	58	61	73	100																													
<i>Micrococcus lysodeikticus</i>	18	65	61	69	61	58	70	62	67	64	62	70	55	58	65	74	100																											
<i>Staphylococcus roseus</i>	21	63	64	63	64	73	76	78	69	75	65	63	67	62	63	66	50	100																										
<i>Micrococcus rubens</i>	23	56	60	51	57	64	67	64	60	66	60	51	55	58	51	53	43	73	100																									
<i>Sarcina erythromyza</i>	24	58	59	53	56	64	66	63	60	65	63	58	58	57	54	51	41	82	76	100																								
<i>Micrococcus roseus</i>	22	59	56	54	52	53	63	61	53	55	49	54	48	47	54	66	49	70	69	68	100																							
O in DNA within the range 67.5-69.5 moles %																																												
<i>Micrococcus candidus</i>	28	54	55	53	58	66	57	66	59	64	59	57	57	56	57	55	42	63	53	52	49	100																						
<i>M. citreus</i>	29	55	56	54	59	63	58	60	56	65	56	54	58	57	54	49	43	57	51	47	79	100																						
<i>Staphylococcus lactis</i>	30	54	55	53	58	52	57	58	55	60	58	53	54	53	50	40	62	58	61	52	76	73	100																					
<i>Sarcina ventriculi</i>	25	57	58	53	55	59	61	59	59	57	59	50	54	53	50	55	42	67	63	69	59	64	65	80	100																			
<i>M. conglomeratus</i>	27	58	59	56	59	63	62	68	61	59	61	59	61	53	59	51	60	49	55	55	62	60	50	62	100																			
<i>Sarcina lutea</i>	2	17	63	67	58	67	72	75	73	68	78	73	67	70	73	71	61	59	54	58	63	56	100																					
<i>S. aurentiaca</i>	2	19	47	49	46	49	50	60	53	53	55	45	50	44	43	46	52	59	43	46	38	41	36	40	35	36	47	50	100															
O in DNA within the range 65.8-67.0 moles %																																												
<i>Micrococcus cyaneus</i>	3	20	64	65	65	74	73	70	70	71	62	60	60	59	60	58	51	75	70	65	59	57	52	60	57	53	66	48	100															
<i>M. luteus</i>	3	14	65	66	69	61	62	65	67	62	72	76	55	58	65	74	74	58	50	53	58	49	43	46	49	51	58	44	51	100														
<i>M. dentrificans</i>	3	26	58	55	53	51	60	58	55	51	53	59	53	47	52	49	54	39	60	68	63	69	51	46	54	62	50	60	36	61	56	100												
O in DNA = 54.2 %																																												
<i>Sarcina oliva</i>	4	31	44	40	40	37	38	44	39	42	39	33	36	33	33	36	44	38	47	38	43	49	50	53	61	56	51	38	45	31	38	100												
O in DNA = 36.4 %																																												
<i>Micrococcus coryliformis</i>	5	35	52	50	48	44	46	43	44	47	40	44	40	40	42	41	52	43	41	42	44	50	62	55	62	56	52	45	28	40	46	48	60	100										
O in DNA 33.3-34.2 %																																												
<i>Micrococcus infans</i>	6	32	39	40	33	38	39	37	37	37	35	32	32	33	35	38	30	41	44	40	46	55	59	63	58	48	44	33	37	30	39	73	72	100										
<i>M. varians</i>	3	34	49	47	41	44	48	52	47	50	49	44	42	41	42	45	45	40	51	45	47	65	63	67	65	52	51	31	46	37	40	67	65	70	100									
<i>M. eurythatis</i>	3	33	40	39	36	33	37	40	35	38	35	33	33	30	29	30	39	30	43	40	42	48	54	55	60	61	54	40	30	35	37	43	61	63	73	70	100							

value = 60 %), 5a and 6a (inter-group *S* value = 67 %), 6a and 4a (inter-group *S* value = 67 %).

The strains of *Staphylococcus aureus* were not subjected to Adansonian analysis.

Table 3. *Intra-group and inter-group S values (% similarity) for all strains used in this study*

		<i>Micrococcus</i>									<i>Staphylococcus</i>			moles %
Sub-groups		1a	1b	2a	2b	2c	3a	3b	3c	4a	5a	6a	GC in DNA	
<i>Micrococcus</i>	1a	72											70.8–73.3	
	1b	59	73											
	2a	56	56	67										
		2b	68	69	58	100*							67.5–69.5	
		2c	50	42	39	50	100*							
		3a	64	67	56	66	48	100*						
		3b	66	55	48	58	44	51	100*				65.8–67.0	
		3c	53	65	53	60	36	61	56	100*				
		4a	39	44	54	38	34	45	31	38	100*			
<i>Staphylococcus</i>	5a	45	44	57	45	28	40	46	46	60	100*		54.2	
		6a	39	45	59	45	31	39	35	41	67	67	71	36.4
														33.3–34.2

* Single strains.

DISCUSSION

The genera *Micrococcus* and *Staphylococcus*. Drawing some general conclusions about the importance of DNA base composition for bacterial taxonomy, Sueoka (1961) pointed out that if the mean moles % GC of the DNA's of two strains is different by 10 % there will be few DNA molecules of the same GC content common to them. Such strains are certainly phylogenetically distant and they should be classified as members of different genera, if it were the aim to include in the same genus only species more or less phylogenetically related. For this reason, the whole set of strains split into two branches obviously representing two distinct genera. The first branch is formed of groups 1, 2 and 3, i.e. strains with a GC content within the range 66.3–73.3 moles %. The second branch is formed of groups 5, 6 and 7, i.e. strains whose GC content ranges from 30.7 to 36.4 moles %. The same division into two branches is confirmed by Adansonian analysis, because all the strains of groups 5, 6 and 7 show a low percentage of similarities with respect to the groups 1, 2 and 3.

Silvestri & Hill (1965) arrived at the conclusion that Gram-positive catalase-positive cocci with low GC moles % values correspond to the genus *Staphylococcus*, while Gram-positive, catalase-positive cocci with high moles % GC values correspond to the genus *Micrococcus*. As outlined by Baird-Parker (1963, 1965) organisms placed in the genus *Staphylococcus* are characterized by ability to grow and produce acid from glucose when incubated under anaerobic conditions in a mineral salts yeast-extract agar medium containing bromocresol purple as pH indicator. On the other hand the genus *Micrococcus* includes cocci which are either unable to produce sufficient acid from glucose to change the indicator bromocresol purple or which grow and produce acid from glucose only in the presence of air. The anaerobic fermentation of glucose is, therefore, recommended as a test for distinguishing the genus

Staphylococcus from *Micrococcus* (Subcommittee on Taxonomy of Staphylococci and Micrococci, 1965; Evans, 1965). Applying this test to the strains studied in this paper we have come to the following conclusions. (1) All the strains showing a high moles % GC were unable to produce acid from glucose under anaerobic conditions. Most strains did not produce any detectable acid from glucose under aerobic conditions. Only some of them produced acid from glucose when grown aerobically (strains of subgroup 2a). (2) The strains (group 7, subgroup 5a) with low values of moles GC content were able to produce acid from glucose anaerobically, while the others (subgroup 6a) only did so aerobically. On the basis of these results the following conclusions may be drawn.

Species of the genus *Staphylococcus* have a GC content within the range 30.7–36.4 moles %. All the Gram-positive cocci so far designated as micrococci and sarcinas which produce acid from glucose when grown under anaerobic or aerobic conditions and which have a GC content within this range should be also classified as *Staphylococcus*. On the other hand species of the genus *Micrococcus* have a GC content within the range 66.3–73.3 moles %. All the Gram-positive cocci hitherto designated as sarcinas and staphylococci which do not produce acid from glucose under aerobic and anaerobic conditions or only aerobically, and which have a GC content in DNA within this range, should be also classified as *Micrococcus*.

The range of GC content as outlined for the genera *Staphylococcus* and *Micrococcus* is only approximate. It will be made more accurate (perhaps a little broader) when more data are available on the DNA base composition of the species belonging to the family Micrococcaceae.

Classification of micrococci

In our opinion it is at present premature to classify micrococci into species until their physiology, biochemistry and DNA base composition are better known. It would be more convenient and less influenced by subjective speculations if for the time being taxonomists aimed to detect among the micrococci natural groups that were clearly defined physiologically as well as biochemically. Such an attempt to classify micrococci has been made by Hill (1959), Pohja & Gyllenberg (1962) and Baird-Parker (1963, 1965). On the basis of DNA base composition the micrococci we have studied may be divided into the following groups and subgroups.

Group 1. This group comprises strains with a GC content within the range 70.8–73.3 %. It consists of two subgroups.

Subgroup 1a is formed mostly of yellow pigmented organisms. Some strains are able to produce both yellow and violet pigments. These strains give yellow pigmented spontaneous mutants which have lost their ability to produce violet pigment and in some media they overgrow the parent culture (Rosypal *et al.* 1963). Thus, the ability to produce yellow pigment appears to be characteristic of all the strains of this subgroup. Non-pigmented strains are exceptions (rare mutants). According to Kocur & Martinec (1962) all the strains included in subgroup 1a do not release detectable acid when grown in peptone water with glucose or other carbohydrates. Results obtained by Baird-Parker (1963, 1965) would indicate that the latter strains can, at least some of them, produce acid when grown in a mineral salts yeast-extract medium containing glucose. In comparison with other subgroups the ability to hydrolyze gelatin and the inability to oxidize mannitol in phosphate buffer at pH 7 may be of some importance for identification of this subgroup.

Considering all the above physiological characteristics, this subgroup 1a appears to correspond to Baird-Parker's *Micrococcus* subgroup 7 (Baird-Parker, 1965). According to Kocur & Martinec (1962) all strains included in subgroup 1a are considered to be identical with *Micrococcus luteus*. However, the type strain *M. luteus* ATCC 398 has a GC content different from that of all the strains of subgroup 1a (see also Rosypalová *et al.* 1966*a, b*). Since the name designation of the type species should not be changed, the name of this subgroup appears to be questionable for the time being.

Subgroup 1b is formed only of pink-pigmented micrococci, which are taxonomically well described. They obviously correspond to Baird-Parker's *Micrococcus* subgroup 8 and to the species named *Micrococcus roseus* (Evans, 1965; Baird-Parker, 1965; Kocur & Martinec, 1962). None of the strains belonging to this subgroup hydrolyze gelatin, but they reduce nitrates and oxidize mannitol and sorbitol in phosphate buffer at pH 7. They are not able to produce acid from glucose in peptone water.

Group 2. The strains belonging to this group have a GC content within the range 67.5–69.5 moles %. The following three subgroups are known:

Subgroup 2a is formed of yellow-pigmented strains. In contrast to other yellow-pigmented cocci they release acid from glucose when grown under aerobic conditions, but no acid is formed when they are incubated anaerobically; they oxidize sorbitol in phosphate buffer at pH 7. By these physiological characters they can be well distinguished from the other subgroups; they correspond to Baird-Parker's *Micrococcus* subgroups 5 and 6. As can be seen from Table 1 and Fig. 2 we included in subgroup 2a the strains which are considered by Kocur & Martinec (1962) as *Micrococcus conglomeratus* and *M. varians*.

Subgroup 2b. This is a yellow-pigmented strain which does not hydrolyze gelatin; in its other characteristics it resembles the strains of subgroup 1a. The similarity of this strain to the strains of subgroup 1a is evident from Fig. 2. The considerable physiological and biochemical similarity of this strain to the organisms of subgroup 1a has resulted in it being considered by Kocur & Martinec (1962) to be identical with the strains of subgroup 1a. The DNA base composition is significantly different from the strains of subgroup 1a, however, and place it in a separate group.

Subgroup 2c. This yellow pigmented strain differs from the strains of subgroup 1a not only in its DNA base composition, but also physiologically and biochemically; see the Adansonian analysis *cf.* Fig. 2.

Group 3. This group comprises strains with a GC content within the range 66.3–67.0 moles %. The species *Micrococcus denitrificans* is not included in this group. Therefore it consists of two subgroups:

Subgroup 3a. This is a violet pigmented coccus not producing a yellow pigment. Although having high *S* values with most strains of subgroup 1a, it differs from them by its DNA base composition and by ability to oxidize mannitol and dulcitol (Rosypalová & Rosypal, 1966; Rosypalová *et al.* 1966*a, b*).

Subgroup 3b. *Micrococcus luteus*, type species, ATCC 398. It has a GC content quite different from the strains of subgroup 1a and cannot be considered identical with them, as believed by Kocur & Martinec (1962). Its classification with the strains of subgroup 1a resulted from its showing some physiological and biochemical similarity to these strains.

'*Subgroup 3c*' formed of *Micrococcus denitrificans* is questionable. Baird-Parker

(1965) suggests that it should be reclassified with the Gram-negative genera and gives the following reasons for it: (1) it is Gram-negative and forms rods in young cultures, (2) it has a cell-wall mucopeptide which contains a wide range of amino acids characteristic of Gram-negative genera. For these reasons *Micrococcus denitrificans* cannot be included in the genus *Micrococcus* even if it has a similar GC content to that of other members of group 3. We must, therefore, correct the opinion of Rosypalová *et al.* (1966*b*), where this species was considered to be closely related to *M. luteus*. On the basis of the above-mentioned facts it shows no phylogenetical relationship to species of the genus *Micrococcus*.

Classification of staphylococci

It is not the aim of the present paper to deal fully with the classification of staphylococci. We touch upon this question only as it concerns the strains originally classified as micrococci and now classified as staphylococci. On the basis of DNA base composition we divided these strains into three groups. *Group 5* is represented by one strain (subgroup 5a) having 36.4 moles % GC. It is the strain *Micrococcus cerolyticus*, ATCC, 12559. Baird-Parker (1965) included it in his *Staphylococcus* subgroup V. It produces acid from glucose when grown under anaerobic conditions. *Group 6* consists of subgroup 6a, i.e. strains with a GC content 33.3–34.2 moles %. Since these strains produce acid from glucose only when grown under aerobic conditions, they have been so far classified as micrococci (*M. varians*). Their GC content places them, however, in the genus *Staphylococcus*.

Group 7 is formed of subgroup 7a and corresponds to the species *Staphylococcus aureus* (Baird-Parker's *Staphylococcus* subgroup I), whose GC content has been found to be 30.7 to 32.7 moles %. On the whole, it may be said that the range of GC content in DNA of the strains belonging to the genus *Staphylococcus* is so large that it will comprise more than the two species, so far recognizable, *Staphylococcus aureus* and *S. epidermidis*. This is also suggested by Baird-Parker's classification, in which staphylococci are divided into six subgroups (Baird-Parker, 1963, 1965). *Group 4* is formed of subgroup 4a represented by a strain with 56.4 moles % GC. It deserves particular attention because its DNA base composition is quite unlike that which has been found with the genera *Micrococcus* and *Staphylococcus*. It produces acid from glucose only when grown under aerobic conditions and is sensitive to several staphylococcal phages, but in DNA base composition it differs from both *Staphylococcus* and *Micrococcus*.

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