Taxonomy of the Staphylococci

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INTRODUCTION

Staphylococci provide a good model to illustrate the different facets and problems of bacterial taxonomy in all three of its parts: classification (the definition of groups and the relationships between these), nomenclature (the naming of the groups), and identification (allocation of new isolates to one or other of the previously defined and named groups). 'Gram-positive, catalase-positive cocci' includes, on the one hand, a group that bacterial taxonomists over the years, and still today, would consider a 'good species', *Staphylococcus aureus*. With minor differences of opinion regarding its delimitation, there is little confusion as to what that name, or its synonyms, is meant to convey, and only exceptional cases of difficulty in identification. On the other hand, we have paradoxically an abundance of taxonomic controversy with regard to the non-*Staphylococcus aureus* organisms, with some classification schemes dividing these into many, very many, species, other schemes recognizing only a few; schemes that place all such organisms into one genus only (usually, but not always, *Micrococcus*) in contrast to others which recognize two or more genera (*Sarcina, Rhodococcus, Gaffkya, Planococcus, Planosarcina,* for example, together with *Staphylococcus* and *Micrococcus*). The nomenclature of the non-*Staphylococcus aureus* organisms is a veritable battlefield littered with hundreds of dead bodies, and identification problems abound at both generic and specific taxonomic ranks.

The history of the taxonomy of staphylococci and micrococci might appear to be more an illustration of the differences between taxonomists as 'splitters' or 'lumpers' and the influences their main occupations exerted on their, usually secondary, taxonomic activities! However, the function of taxonomy and the job taxonomists set themselves to do is to compile, for the benefit of everyone, an information storage and retrieval system to reflect at any one time the current state of knowledge. Taxonomists

themselves do not always realize or appreciate that when they classify bacteria they are not really classifying the bacteria themselves but rather our knowledge of them (Rescigno & Maccacaro, 1961). As new information is gained, taxonomists would be failing in their duty to fellow, but not taxonomically-minded, colleague microbiologists if they failed to revise the information storage/retrieval system that is called 'classification' to take into account that new information. A classification is only an hypothesis of the best way to represent the similarities and dissimilarities of nature. As any other scientific hypothesis, a classification will be replaced by a new hypothesis, if and when the old system no longer reflects the facts as they are known. Thus, the development of staphylococcal taxonomy is not merely the result of the whims and fancies of individual taxonomists playing some mysterious game of their own. Of course, these scientifically laudable aims were not always the root cause of changes; some may well have been 'whims and fancies'.

The more recent history of staphylococcal taxonomy does also include the application of several methods or approaches that have had major impact on the whole practice of bacterial taxonomy.

Early History, 1882–1923

A number of workers in the 1870s had observed cocci in inflammations, abscesses and pus and Billroth (1874) thought they were all one organism, *Coccobacteria septicum*. It was by no means accepted that these cocci were the etiological agents of disease conditions and it was Ogston's work that showed that they were. With regard to taxonomy, Ogston was the first to recognize two kinds of cocci, those in chains and those in groups. He used Billroth's name, *Streptococcus*, for the chain-forming cocci, and introduced the name *Staphylococcus* for the group-forming cocci. Unfortunately and unwittingly, Ogston (1882) fell foul of one of the rules of the game, not a difficult thing to do since none existed at that time! Botanists assumed early jurisdiction over the nomenclature of bacteria, claiming they were plants and it was not until about 1936 that the few bacteriologists with influence on the botanical committee which dealt with bacterial nomenclature began to develop a separate Bacteriological Code of Nomenclature. A drafting committee was set up in 1939, reported in 1947 and the first Bacteriological Code was published in 1948. (Incidently, bacteria were not deleted from the Botanical Code until as recently as 1975.) In the 1880s, and a long time afterwards, the people interested in staphylococci did not feel beholden to the Botanical Code and as medical micro-

biologists indeed why should they have been? It is more than forgivable then that Ogston broke a Rule of Nomenclature that, for bacteria, was to come into effect, and with retrospective action, some sixty-six years later! The Rule in question (Rule 13 of the 1958 edition, Rule 27 of the 1976 edition of the Bacteriological Code) says that a name of a genus is not validly published if it is unaccompanied by a description of the genus or a citation to a previously published description.

Rosenbach (1884) gets the credit for the genus name *Staphylococcus* and the species name *S. aureus* for he, equally unwittingly, did not transgress this or, apparently, other Rules. Rosenbach demonstrated two differently coloured forms which he named *Staphylococcus pyogenes aureus* and *S. pyogenes albus* and Passet (1885) added *S. pyogenes citreus*. I find it fascinating to note that while successive classification schemes from this period were forced, through lack of other data, to frequently use pigmentation as a key character, in the case of staphylococci, these early workers realized how untrustworthy it is as a diagnostic character, as evidenced by the use of trinomials by Rosenbach and Passet.

Although Ogston failed to anticipate that a Bacteriological Code would be approved years later, and Rosenbach was equally ignorant but in better luck, the name *Staphylococcus* was not readily accepted. Cohn (1872) is credited with the name *Micrococcus,* much used colloquially in Ogston's writings too, and most early classifications, up to 1923, did not recognize a genus *Staphylococcus* as distinct from *Micrococcus*.

Buchanan (1925) provides the most authorative summary of early bacterial classifications, presenting these in the form of diagnostic keys with the distinguishing characters, down to genus level. Using 1882 as a starting point, and 1923, the year of the first edition of *Bergey's Manual,* as an endpoint, some 25 systems did not recognize *Staphylococcus* and only 9 did (see table 1). Some authors (e.g. Migula, Flügge) appear in both halves of table 1 and, at the same time, those listed classifications were only those that attempted to be comprehensive classifications of all bacteria. In brief, the formal classifications and most writings of this period, clearly show a body of opinion against separation of *Staphylococcus* from *Micrococcus*. However, significant among those supporting *Staphylococcus* were the Winslows (1908), Buchanan (1917) and The Society of American Bacteriologists Committee (1917, 1920). The SAB Committee was very influential in the formation of *Bergey's Manual,* the first edition of which and successive editions, with the exception of the sixth, all recognized *Staphylococcus*. The SAB Committee must have been, in turn, influenced by Winslow's and Buchanan's presence on it; a case of the right men on the right committee at the right time?

There were two major considerations affecting these early classifications: a preoccupation with distinguishing between pathogenic and saprophytic organisms, and with the benefit of hindsight, undue importance being accorded to pigmentation again. More important than the inclusion of *Staphylococcus* into *Micrococcus* was a risk of losing the identity of a perfectly good species, by whatever name, and thus in Migula (1900), Rosenbach's *Staphylococcus pyogenes albus* became *Micrococcus pyogenes; S. pyogenes aureus* became *M. aureus* and Passet's *S. pyogenes citreus* became *M. citreus*. Winslow & Winslow (1908) divided *Staphylococcus* into two, apparently, subgenera: orange strains in *Aurococcus* and white ones in *Albococcus*.

TABLE I

Recognition, or not, of *Staphylococcus* in early bacterial classifications (from Buchanan 1925)

(a)	Did not accord recognition:	
	Van Tieghem, 1884	Chester, 1897, 1901
	Zopf, 1885	Kendall, 1902
	Flügge, 1886	Matzuchita, 1902
	Schroeter, 1886	Conn, 1909
	Maggi, 1887	Orla-Jensen, 1909
	Blaumgarten, 1890	Heim, 1911
	Ludwig, 1892	Engler, 1912
	Sternberg, 1892	Meyer, 1912
	Migula, 1894, 1900	Löhnis, 1913
	Lehmann & Neumann, 1896	Vuillemin, 1913
	Fischer, 1897, 1903	Castellani & Chalmers, 1919
(b)	Did accord recognition:	
	Trevisan, 1887	Flügge, 1907
	De Toni & Trevisan, 1889	Winslow & Winslow, 1908
	Cornil-Babes, 1890	Buchanan, 1917
	Migula, 1890	Soc. Am. Bact., 1917, 1920

The early classifications were, of course morphological (excepting Orla-Jensen, 1909). Starting in the 1880s with such characters as cell shape, presence or not of cysts or spores, planes of cell division and soon adding motility, presence of sulphur, life-cycles, sheaths to filaments, capsules,

pigmentation and some colonial features, by the 1900s these were supplemented with swarming, polar or peritrichous flagella and aerobe *v.* anaerobe. Although the amount of information was limited, these few characters can, and were, arranged in many different combinations; hence the diversity in the classifications.

Those classifications that did recognize *Staphylococcus* did not do so very convincingly! Thus, De Toni & Trevisan (1889), within a division of cocci not in cysts, capsules or sheaths, divided cocci into pairs (*Neisseria*), 'botryoid masses' (*Staphylococcus*), and occurring singly or in amorphous masses (*Micrococcus*). Migula (1890) distinguished between cocci that separated after division (*Micrococcus,* spelt incidently with a 'k': *Mikrococcus*), and those that did not; of the latter those in irregular groups were *Staphylococcus*, in chains *Streptococcus*, packets *Sarcina,* flat plates or tetrads *Merismopedia*, gelatinous masses *Leuconostoc.* Flügge's (1907) classification features the gram reaction, but in his system and some subsequent ones this seems to confuse some aspects while serving to separate away *Neisseria!* In Flügge then, cocci in chains, gram-positive were *Diplococcus* and *Streptococcus*; in packets, gram-negative, *Sarcina*; elongated cells, gram-negative were *Micrococcus* of the *Diplococcus* type; in twos or fours, of the *Tetragenus* type; irregular masses, of the *Staphylococcus* type; it would appear that 'Staphylococcus type' and the others indicate a subgeneric division. The Winslows' (1908) first division was into parasites or saprophytes; parasites in pairs were *Diplococcus*; chains in zoogloeal masses, *Ascococcus*; chains were *Streptococcus*; irregular groups *Staphylococcus*; whilst saprophytes in irregular groups were *Micrococcus*. Buchanan (1917) to some extent followed the Winslows: parasites in pairs were either *Diplococcus* (gram-positive) or *Neisseria* (gram-negative); irregular groups *Staphylococcus*; while saprophytes or, intriguingly 'facultative parasites', in packets were *Sarcina,* not in packets were *Micrococcus* (usually yellow) or *Rhodococcus* (red). The Society of American Bacteriologists Committee (1917) put a first division on red (*Rhodococcus*) or not, then with the latter on gram reaction and under gram-negative listed *Neisseria* (pairs), *Saracia* (packets) and *Micrococcus* (not in packets)! The gram-positives were divided into *Streptococcus* (chains), *Staphylococcus* (in groups, orange pigmentation), and *Albococcus* (in groups, white). Some amends were made with regard to the gram reaction of *Micrococcus* in the SAB Committee 1920 scheme, but this also went back to a first division into parasites and saprophytes: parasites occurring as flattened coffee-bean pairs and gram-negative were *Neisseria*, not flattened and gram-positive were *Diplococcus* (pairs), *Streptococcus* (chains), and *Staphylococcus* (irregular groups); saprophytic organisms were *Leuconostoc, Sarcina*

(packets), *Micrococcus* (yellow) and *Rhodococcus* (red). Thus *Micrococcus* was not recorded as gram-negative, but nor was it recorded as gram-positive, for the gram reaction does not feature in the saprophytic section of the key to Coccaceae!

Essentially then, in this early period, the distinction between *Staphylococcus* and *Micrococcus* was very tenuous indeed, even for those who were in any case in a minority of wishing to make the distinction at all. At the same time, it was popular to make divisions on pigmentation and consequent subdivisions within what is now known as *S. aureus*. Another aspect, that would be tedious in the extreme to detail, was that despite few characters, the numbers of species recognized was extraordinary. Simply as indicators; Flügge (1890) recognized three species in *Staphylococcus*, 33 *Micrococcus* spp., and 3 *Sarcina* spp., but Migula (1900), 201 *Micrococcus* spp., and a further 27 partly described, 55 *Sarcina* spp., 7 *Planococcus* spp., and 3 *Planosarcina*.

In this early period, mention must be made of Andrewes & Gordon (1907) as pioneers in proposing a biochemical classification of human staphylococci. They recorded pigmentation and checked pathogenicity to guinea pigs, recognizing four species: *S. pyogenes* (orange, pale yellow or white, highly pathogenic), *S. epidermidis albus* (white, feebly pathogenic), *S. salivarius* and 'Scurf staphylococci' (both white, non-pathogenic). Cowan (1962) gives a table with seven biochemical tests Andrewes & Gordon used: clot in milk, gelatin liquefaction, nitrate reduction, and acid from maltose, lactose, glycerol, and mannitol; on these tests the number of differences between the species ranged from just one (mannitol) between *S. pyogenes* and *S. epidermidis albus* up to five between either of those two species and the 'scurf staphylococci'. Cowan goes on to note however that this biochemical classification was not readily accepted, quoting Dudgeon (1908) who found almost as many different combinations of biochemical characters as there were strains and Cummins & Cumming (1913) who found acid from carbohydrates to be unreliable when re-tested some months after initial examination.

BERGEY'S MANUALS, 1923–1957

The report of the SAB Committee (1920) provided the stimulus for the publication in 1923 of a comprehensive manual for the identification of bacteria, 'arranged' by an SAB Committee of five members, with David H. Bergey as chairman, known as *Bergey's Manual of Determinative Bacteriology*. Further editions appeared (2nd—1926; 3rd—1930; 4th—1934) in

which authorship was attributed as 'by D. H. Bergey, assisted by . . .' (an SAB Committee); the 5th edition (1939) was authored by Bergey, Breed, Murray & Hitchens 'assisted by' 25 others. After Bergey's death, the Bergey's Manual Trust published further editions (6th–1948; 7th–1957; 8th–1974) under the names of specific Editor-Trustees assisted by an ever growing number of contributors (60, 94 and 137 respectively). These facts are mentioned because *Bergey's Manual*, to quote Cowan (1978), 'introduced a new nomenclature which was resented, disliked, and, outside the US, regarded as an American product'. In fact, a lot of the nomenclature used was European and its post-war authorship has been truly international. Moreover, it is the only book that seriously attempts to cover all bacteria (excepting perhaps Krassilnikov, 1959, in which the nomenclature is not at all in agreement with international opinion) and many of its critics failed to appreciate its main task: to provide keys to identify bacteria. Thus, for relative ease of identification, certain bacteria that one might reasonably think not very closely related in a classification sense were in fact placed rather close to each other.

TABLE II

Numbers of species listed in successive editions of *Bergey's Manual* (Bl-8), *Index Bergeyana* (IB) and Approved Lists (AL)

	B1 1923	B2 1926	B3 1930	B4 1934	B5 1939	B6 1948	B7 1957	IB 1966	B8 1974	AL 1980
Staphylococcus	6	5	5	6	9	—	2	79	3[a]	13
Micrococcus	27	27	41	46	46	22	16	748	3[b]	9
Sarcina	10	10	11	11	14	9	10	133	2	2
Gaffkya	—	2	2	3	4	2	2	7	—	—
Rhodococcus	5	5	6	6	—	—	—	14	—	(10)
Planococcus	—	—	—	—	—	—	—	9	1[c]	2

[a] 1 further species listed *Species incertae sedis*
[b] 6 further species listed *Species incertae sedis*
[c] 7 further species listed *Species incertae sedis*

Table II summarizes the numbers of species that successive editions of *Bergey's Manual* recognized, with regard to *Staphylococcus, Micrococcus* and like organisms. Between the 7th and 8th editions, the Bergey's Manual Trust authorized the publication of *Index Bergeyana,* which was simply a

listing of all bacterial names on record (whether used by *Bergey's Manual* or not), with statements concerning their nomenclatural status. Most names that have appeared in the literature reduce to synonyms and table II also gives the figures for *Staphylococcus,* etc., taken from *Index Bergeyana.* The last column of table II refers to the Approved Lists published in 1980 and which will be discussed later.

From table II, it is evident that pre-war (1st–5th eds.) there was a steady increase in numbers of species recognized, to a maximum 9 staphylococci and 46 micrococci in 1939, and post-war a steady decline to a record low number of only 3 species each fully described in the 8th (and current) edition. *'Species incertae sedis',* means species of uncertain affiliations and, in the case of staphylococci and micrococci accorded only a few lines of text.

TABLE III

Bergey's Manual, 1st ed., (1923)

(a) *Genera of family Coccaceae:*

Tribe	Genera	No of species
Neisseriae	*Neisseria*	7
Streptococceae	*Diplococcus*	1
	Leuconostoc	3
	Streptococcus	24
	Staphylococcus	6
Micrococceae	*Micrococcus*	27
	Sarcina	10
	Rhodococcus	5

(b) *Species of genus Staphylococcus:* (all 6 pathogenic)

Orange: Lactose +, Gelatin +			*S. aureus*
Lemon: Lactose +, Gelatin +			*S. citreus*
White: Lactose +, Gelatin +:			
Sucrose	Mannitol	Raffinose	
+	−	−	*S. epidermidis*
+	+	−	*S. albus*
+	+	+	*S. pharyngis*
White: Lactose +, Gelatin −			*S. tetragenus*

Tables III–VII and IX summarize the taxonomic structures the successive editions of *Bergey's Manual* used for the family *Coccaceae* (1st–4th eds.) or *Micrococcaceae* (5th *et seq* eds.), and outline how the species of *Staphylococcus* was identified. In 1923, *Staphylococcus* was placed in the

Tribe (a subdivision between family and genus taxonomic ranks) Streptococceae, but in 1926 already moved to Tribe Micrococceae. The recognition of staphylococcal species was primarily on pigmentation and secondarily on acid produced from a few carbohydrates and gelatin liquefaction. The last species listed in the first edition *(S. tetragenus)* was subsequently moved to a new genus *Gaffkya* in the Tribe Neisseriae (2nd, 3rd eds.), which genus was later moved from there to the Tribe Micrococceae (4th ed.). The 4th edition also introduced into its diagnostic key a type of feature (the source from which an organism had been isolated) that most users of *Bergey's Manual* justly criticized. In this staphylococcal instance, subsequent to identification on the basis of sucrose, mannitol and raffinose, the only distinction between *S. muscae* and *S. albus* was that the former was isolated from house-flies, the latter from human skin and mucous membranes! House-flies are known regrettably to occasionally perambulate on human skin.

TABLE IV

Bergey's Manual, 2nd (1926), 3rd (1930), and 4th (1934) eds

(a) *Genera of family Coccaceae:*

Tribe	Genera	No. of Species: 1926	1930	1934
Streptococceae	*Diplococcus*	3	3	3
	Streptococcus	25	35	31
	Leuconostoc	3	4	3
Neisseriae	*Neisseria*	7	7	8
	*Gaffkya**	2	2	3
Micrococceae	*Staphylococcus*	5	5	6
	Micrococcus	27	41	46
	Sarcina	10	11	11
	Rhodococcus	5	6	6

(b) *Species of genus Staphylococcus:*

As for 1st ed (see table III), except:

(i) *S. tetragenus* moved to *Gaffkya tetragena*

(ii) 4th ed., add *S. muscae* (from house flies), same key characters as *S. albus* (from skin and mucous membranes)

* *Gaffkya* moved to tribe Micrococceae in 4th ed.

Moving from the keys to the descriptions, in these early editions, for *S. aureus* the microscopical appearance and gram reaction were given together

with appearance and action on gelatin stab; colonial appearance on agar plate and slope; appearance in broth; action on litmus milk; appearance on potato; indole, nitrates, H_2S results; acid (or not) from six carbohydrates; and statements of pathogenicity, aerobic culture, optimum temperature and habitat. By the 4th edition the statement 'Ammonium salts are not utilized' was added, a test introduced by Hucker (1924) and which became a key character in the 6th edition (1948) to identify the staphylococcus-like organisms from micrococci (in the 6th edition, the genus *Staphylococcus* is merged with *Micrococcus*). This test might be the first example of attempts to find a single, reliable, biochemical character to separate *Staphylococcus* from *Micrococcus*; there have been several, failed attempts since.

TABLE V

Bergey's Manual, 5th ed., (1939)

Note: *Streptococcus* etc. moved to Lactobacteriaceae
Neisseria etc. moved to Neisseriaceae
Rhodococcus moved to *Micrococcus*

(a) *Genera of family Micrococcaceae:*

Micrococcus	46 spp.
Staphylococcus	9 spp.
Gaffkya	4 spp.
Sarcina	14 spp.

(b) *Species of genus Staphylococcus:*

Aerobes to facultative anaerobes: 6 spp. as in *Bergey's Manuals* 2nd-4th eds (see table IV)

Anaerobes, all from human sources:

Gas from peptones: fetid odour	*S. asaccharolyticus*
: no fetid odour	*S. aerogenes**
No gas from peptones	*S. anaerobius*†

* Pathogenic † Pathogenic to guinea-pigs and rabbits

The 5th edition (1939) made substantial improvements at the higher ranks, giving greater emphasis to the differences between streptococci, neisserias and *Staphylococcus-Micrococcus* by deleting Tribes and creating separate families for the former two and introducing the family Micrococcaceae. The identification key to species of *Staphylococcus* remained

unchanged, however, as did also the species descriptions but for the addition starch hydrolysis; three anaerobic species were added.

The late 1930s saw a re-discovery of coagulase as a test for pathogenic staphylococci (Loeb, 1903; Much, 1908; then largely overlooked until Walston, 1935; Fisher, 1936; Cruikshank, 1937; Chapman *et al.*, 1937, 1938; Blair, 1938), but this important test was omitted from the 5th edition, appears in the species descriptions of *Micrococcus pyogenes* var *aureus* and var *albus* in the 6th edition (1948), and became a key character in the 7th edition (1957). In the proper endeavour over the years to identify pathogenic staphylococci greater attention had been paid to correlations between presumed pathogenicity and mannitol fermentation, or with pigmentation of course, or gelatin liquefaction, all duly reflected in *Bergey's Manuals* 1–6; and also with haemolysins, leucocidin and serological differences, with which *Bergey's Manuals* failed to keep up-dated until the 7th edition.

TABLE VI

Bergey's Manual, 6th ed., (1948)

Note: Staphylococcus moved to *Micrococcus*

(a) *Genera of family Micrococcaceae:*

Micrococcus		22 spp.
Appendix A	*Methanococcus*	1 sp.
Appendix B	*Pediococcus*	1 sp.
Gaffkya		2 spp.
Sarcina		9 spp.

(b) *Species of genus Micrococcus:*

Aerobes to facultative anaerobes		
No pink pigment		
NO_3 reduction –		5 spp.
NO_3 reduction +		
$NH_4H_2PO_4$ utilization +		3 spp.
$NH_4H_2PO_4$ utilization –		
Gelatin + Mannitol +		
Orange	*Micrococcus pyogenes aureus*	
White	*Micrococcus pyogenes albus*	
Yellow	*Micrococcus citreus*	
Gelatin –		2 spp.
Pink pigment		5 spp.
Anaerobic		5 spp.

The merger of *Staphylococcus* into *Micrococcus* in the 6th edition spurred disbelievers into new efforts to separate the two and caused a rash of 'lumpers' classifications! Thus, Abd-el-Malek & Gibson (1948) used some 18 tests (799 strains, heavily biased towards dairy strains) to form a classification that recognized three main divisions: Staphylococcus Group (four subgroups distinguished on ammonia produced from arginine, acetoin production, coagulase), an Intermediate Group (no acid from glucose, moderately thermoduric) and a Dairy Group (acid from glucose and thermoduric, subdivided into two subgroups distinguished by acid from glycerol). Evans (1947, 1948) observed that the correlation between acid from mannitol and coagulase production was greater if the first test was carried out anaerobically and then went on (Evans, Bradford & Niven, 1955) to devise a glucose containing medium by which staphylococci could be separated from micrococci: they proposed that *Staphylococcus* be used for those organisms that could grow and produce acid (from glucose) anaerobically; *Micrococcus* for those that could not. Shaw, Stitt & Cowan (1951) enlarged the horizons by using some 37 tests (402 strains) and tried several re-arrangements of data until they were 'satisfied' with a classification to just five species (all considered *Staphylococcus*, not *Micrococcus*) on the basis of coagulase (positive: *S. aureus*), glucose fermentation (negative: *S. afermentans*), acetoin production (positive: *S. saprophyticus*), pink pigment (positive: *S. roseus,* negative *S. lactis*). These groupings were put forward because on the basis of the other tests not used in the key each group appeared homogeneous. They described their classification as 'arbitrary and artificial' and Cowan (1962) himself later quoted it as a good example of bad taxonomy. Some time after their proposal, Hill (1959) using the then novel technique of numerical taxonomy, which can be considered a more refined way of devising homogeneous (or relatively homogeneous) groups over many characters, showed that their *S. aureus, S. saprophyticus* and *S. roseus* were indeed 'good' species, but strains of the other two species were sufficiently different from each other as to each merit a species rank, if *S. aureus* was to be taken as indicative of species rank.

The stage was thus set for a notable change between the 6th and the 7th editions of *Bergey's Manual* (see table VII). The family Micrococcaceae was revised by Breed, but recognized Evan's generic distinction, thus 'Action on glucose, if any, is oxidative. Aerobic' lead to *Micrococcus* and 'Glucose fermented anaerobically with the production of acid. Facultatively anaerobic' lead to *Staphylococcus*. *Micrococcus* was revised by Hucker and Breed (and they revised respectively *Gaffkya* and *Sarcina*) and shows little change from the 6th edition, but *Staphylococcus* was revised by Evans

himself, resulting in just the two species, *S. aureus* and *S. epidermidis,* on the basis of mannitol and coagulase. A footnote warns that the name 'S. albus' should never be used.

TABLE VII

Bergey's Manual, 7th ed., (1957)

(a)	*Genera of family Micrococcaceae:*			
	Aerobes/fac.			
	anaerobes:	Irreg. groups, oxidative	*Micrococcus*	16 spp.
		Irreg. groups, fermentative	*Staphylococcus*	2 spp.
		Tetrads	*Gaffkya*	2 spp.
		Packets	*Sarcina*	10 spp.
	Anaerobes		*Methanococcus*	2 spp.
		and	*Peptococcus*	11 spp.
(b)	*Species of genus Staphylococcus:*			
	Mannitol +, Coagulase +		*Staphylococcus aureus*	
	Mannitol −, Coagulase −		*Staphylococcus epidermidis*	

PROGRESS BETWEEN *Bergey's Manual* 7TH EDITION (1957) AND 8TH EDITION (1974)

Staphylococcal taxonomy had thus become extremely simple: glucose fermentation to distinguish between *Staphylococcus* and *Micrococcus* and coagulase to differentiate *S. aureus* and *S. epidermidis* (frequently *'S. albus'* in the medical literature). Reliance on one character only for generic and, within *Staphylococcus*, specific differentiation must have appeared very convenient and practical but, in fact, was not good taxonomic practice in that this was over-simplification and failed to reflect the known difficulties in application of the generic distinction and heterogeneity in the non-*S. aureus* strains. The glucose fermentation test was not standardized and so, depending on the precise method of determination, some strains could be allocated to either genus and yet others gave such weak positive results that they would be called 'intermediates'. Absolute divisions on the basis of one or a few tests rarely stand the test of time in microbiology; Lucas & Seeley (1955) reported a catalase-negative strain of *M. pyogenes* var *aureus*; coagulase negative variants of *S. aureus* are known.

In the same year of publication of the 7th edition of *Bergey's Manual,* Sneath (1957a,b) introduced some new concepts to the whole practice of microbial taxonomy, the techniques at first known as Adansonian

classification which developed into the discipline of numerical taxonomy. In its essence, the philosophy of numerical taxonomy is that taxonomic groups should be formed on the basis of overall similarity between strains, considered over as wide a range of properties as practicable. *A priori,* different properties should be given all the same weight or importance (thus, numerical taxonomy moves away from the idea that one can choose *a priori* which are the important characters), which then enables the use of mathematical–statistical methods directly or indirectly to establish correlations between properties. The final classification process (definition of groups and relationships between these) is made on the basis of highly correlated characters and with reference to a numerical similarity scale. Another important aspect is that groups so defined are 'polythetic' groups (organisms of a polythetic group will have most of their characters in common, but no one single character need necessarily be common to all), as opposed to 'monothetic' groups which usually result from the traditional method of classification (a monothetic group is defined by a character or set of characters and all organisms in the same group must possess all the defining characters).

Hill (1959) applied this method to a rather small selection of strains and showed that *S. aureus* was a homogeneous 'good' species, the *S. saprophyticus* of Shaw, Stitt & Cowan (1951) was also acceptable but not so homogeneous as *S. aureus,* the whole group could be divided into two genera, and that in the *Micrococcus* half, *M. roseus* was a 'good' species. This was the result of using common, widely-used tests. There were several strains (*S. lactis* and *S. afermentans* in the system of Shaw *et al.*) which individually merited species rank.

The next substantial and influential study in staphylococcal taxonomy was that of Baird-Parker (1963, 1965), who used a far greater number of strains, and tests, but did not use numerical taxonomy techniques to analyse the data. Baird-Parker used the anaerobic glucose utilization test (a modified Hugh & Leifson, 1953, test) to divide staphylococci from micrococci and defined six subgroups within *Staphylococcus* and eight within *Micrococcus*; see table VIII. *Staphylococcus* subgroup I corresponds to *S. aureus*, subgroups II–V *S. epidermidis,* subgroup VI was a new group. There were some parallelisms between subgroups in the two genera; thus some strains of *Staphylococcus* subgroup IV were different from *Micrococcus* 1 only on the basis of anaerobic glucose utilization. Subsequently, reports began to appear of micrococci identified according to the Baird-Parker scheme, isolated from clinical sources, especially *Micrococcus* subgroup 3, and doubts arose as to whether Micrococcus subgroups 1–4 were in fact staphylococci when tested by the standard method for deter-

mining anaerobic utilization of carbohydrates put forward by the ICSB Subcommittee on Taxonomy of Staphylococci and Micrococci (Subcommittee, 1965), or other variants of the test.

TABLE VIII

Baird-Parker's Classification (1963, 1965)

Anaerobic glucose utilization, positive: *Staphylococcus*
negative: *Micrococcus*

(a) *Staphylococcus*

	Subgroups I	II	III	IV	V	VI
Coagulase	+	−	−	−	−	−
Phosphatase	+	+	+	−	−	−
Acid from mannitol: O_2	+*	−	−	−	−	+
AnO_2	+*	−	−	−	−	−*
Acetoin	+	+	−	+	+	+
Acid from: lactose	+*	+*	v	−	+	v
maltose	+	+	−	v	+	v
Growth at 10°	+*	−	−	−	−	−*

(b) *Micrococcus*

	Subgroups 1	2	3	4	5	6	7	8
Acid from glucose, O_2	+	+	+	+	+	+	−	−
Phosphatase	−	−	−	−	−	+	−	−
Acetoin	+	+	+	+	−	−	−	−
Terminal pH, glucose broth	4.6	5.1	5.0	5.2	5.5	5.3	6.5	6.2
Acid from: arabinose	−	−	−	+	v	+	−	−
lactose	−	+	v	+	+*	+	−	−
maltose	v	+	+*	+	+*	+	−	−
mannitol	−	−	+	+	+*	+	−	−
Lipolysis	v	+	+	−	−*	+*	v	−
Tween hydrolysis	−*	−	v	−	−	+	v	v
Growth at 10°	−*	+*	+*	+	+*	+	+*	+
Red pigment	−	−	−	−	−	−	−	+

v = variable * usual result

Lee, Wahl & Barbu (1956) were the first to propose that DNA base composition, usually expressed as per cent guanine and cytosine (per cent GC), could be useful taxonomically. Some molecular biologists, in a first wave of enthusiasm, proclaimed that they would soon supplant all

traditional, or indeed also numerical, taxonomy through the study of the differences and similarities between the DNA of micro-organisms. The thesis was that the DNA contains encoded in base triplets all the information necessary to 'make' a microbe; traditional, or even numerical, taxonomists were studying only phenotypic traits corresponding to only a small portion of the total genome. Whilst this ousting has not taken place, the use of DNA data, especially per cent GC and *in vitro* molecular hybridization techniques, has had a revolutionary effect on bacterial taxonomy. With regard to per cent GC, this is only useful taxonomically in a negative sense: if DNA samples from different organisms have widely different base compositions, it follows that their base sequences (i.e. genetic information) must be different and the taxonomic conclusion to draw is that the organisms are unrelated. Belozersky and Spirin (1960) reported various strains of *S. aureus* and one of *S. epidermidis* had DNA base compositions in the range of 31–40 per cent GC, but strains of *Sarcina lutea* and one of *M. lysodeikticus* (both synonyms of *M. luteus,* the type species of *Micrococcus*) were very different: 64–74 per cent GC. Silvestri & Hill (1965) followed this up with base composition determinations of several strains that had been used in Hill's previous numerical taxonomy study; these results showed a complete correlation between the allocation of strains to *Staphylococcus* or *Micrococcus* and base composition. Two strains of 'S. lactis' that had been placed in *Staphylococcus* had low per cent GC composition, and one allocated to *Micrococcus* had a high per cent GC composition. This finding was confirmed and extended by many other workers and, moreover, some strains which, in the Baird-Parker system, would be considered *Micrococcus* were found to have staphylococcal-like base compositions.

The range in base compositions is almost as great as the total range known to occur in bacteria, which is 25 per cent GC for certain mycoplasmas and clostridia, to 75 per cent GC for micrococci and streptomycetes. With such a big difference then between *Staphylococcus* and *Micrococcus*, it appeared that surely the existing problems of separating the two genera on the basis of anaerobic glucose utilization were resolvable! For practical reasons, the glucose test could not be simply replaced with the determination of per cent GC, as not all laboratories and certainly not clinical laboratories were able, or equipped, to determine base compositions. Comparisons were made of different methods of determining carbohydrate fermentation in an endeavour to obtain the best correlation with per cent GC results (Mortensen & Kocur, 1967; Kocur & Mortensen, 1967), but these attempts have never been wholly successful; there always seem to be exceptional, recalcitrant strains.