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Agreement Between Deoxyribonucleic Acid Base Composition and Taxometric Classification of Gram-Positive Cocci[1]

L. G. SILVESTRI AND L. R. HILL

Progetto Sistematica Actinomiceti, Istituto "P. Stazzi," Università Statale, Milan, Italy

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ABSTRACT

SILVESTRI, L. G. (Università Statale, Milan, Italy), AND L. R. HILL. Agreement between deoxyribonucleic acid base composition and taxometric classification of gram-positive cocci. J. Bacteriol. **90**:136–140. 1965.—It had been previously proposed, from taxometric analyses, that gram-positive, catalase-positive cocci be divided into two subgroups. Thirteen strains, representative of both subgroups, were examined for deoxyribonucleic acid (DNA) base composition, determined from melting temperatures. Per cent GC (guanine + cytosine/total bases) values fell into two groups: 30.8 to 36.5% GC and 69 to 75% GC. Strains with low per cent GC values belonged to the *Staphylococcus aureus–S. saprophyticus–S. lactis* taxometric subgroups, and those with high per cent GC values belonged to the *S. roseus–S. afermentans* subgroup. The hypothetical nature of any classification is emphasized, and, in the present work, the hypothesis derived from taxometric analyses of division into two subgroups is confirmed by the study of DNA base ratios. The two subgroups correspond, respectively, to the genera *Staphylococcus* and *Micrococcus*.

The introduction to bacterial taxonomy of new methods, exploiting, on one hand, electronic computers to process large quantities of data and, on the other hand, recent advances made in molecular biology, has led to a renovated formulation of taxonomy. Discussions of the newer schools of thought in taxonomy will be found in the books by Sokal and Sneath (1963) and Davis and Heywood (1963). We interpret the essential characteristic of this new formulation to lie in that ". . . taxonomy ceases to be a purely descriptive science. A developmental stage would be reached in which previously gained knowledge would be utilized to formulate hypotheses, to be accepted or rejected according to experimental results" (Silvestri and Hill, 1964). Here, we propose to illustrate how this new formulation can be usefully applied in bacterial taxonomy.

In previous studies (Hill, 1959; Hill et al., 1965), organisms belonging to the genera *Staphylococcus* and *Micrococcus* were studied with different numerical taxonomic (or "taxometric") methods; i.e., they were reclassified according to their overall phenetic similarity, by use of different programs. The principal conclusion drawn was that the organisms could be divided into two major subgroups. According to the nomenclature of Shaw, Stitt, and Cowan (1951), the first subgroup ("*Staphylococcus*") comprises *S. aureus*, *S. saprophyticus*, and *S. lactis*, and the second subgroup ("*Micrococcus*") comprises *S. afermentans* and *S. roseus*.

We have already stated that groups which emerge from taxometric analyses are acceptable only if they are more homogeneous than the original set of organisms (Silvestri and Hill, 1964). Criteria for the definition of "homogeneous" are under study (Rogers and Tanimoto, 1960; Rogers and Fleming, 1964; Silvestri, Hill, and Möller, 1963), but, until this problem has been satisfactorily resolved, we propose to consider group stability (as revealed by repeated formation of the same groups by different taxometric methods) to be indicative of homogeneity (Hill et al., 1965; see also Discussion). A comparison of the taxonomic trees (dendrograms) reported by Hill et al. (1965) indicates that, although the insertion of single strains may differ somewhat from one dendrogram to another, nonetheless the major division is constant and the two subgroups comprise the same strains in the different dendrograms. For this reason, we assume the two subgroups to be stable and most probably homogeneous.

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This division seems to correspond to that of Evans, Bradford, and Niven (1955), who divided gram-positive, catalase-positive cocci into two genera, *Staphylococcus* and *Micrococcus*, on the basis of ability to produce acid from glucose under anaerobic conditions. For further reviews of the taxonomy of these organisms, reference should be made to Kocur and Martinec (1962) and Baird-Parker (1963).

The thesis of a division into two subgroups, probably homogeneous and probably coinciding with the division made by Evans et al. (1955), would be strengthened if further evidence, independent of taxometric analysis, can be found. Such evidence can be supplied by a study of deoxyribonucleic acid (DNA) base ratios, since these represent the basis of the other, molecular, modern approach to bacterial taxonomy (Lee, Wahl, and Barbu, 1956; Marmur, Falkow, and Mandel, 1963).

There are few data in the literature concerning DNA base ratios of these organisms (Table 1) but the existing data indicate fairly clearly two groups: the first with per cent GC (guanine + cytosine/total bases) values in the range of 30 to 40%, comprising strains recognizable as staphylococci, and the second in the range of 64 to 74% GC, comprising strains recognizable as micrococci or sarcinae. [Strains called *M. pyogenes* by Belozersky and Spirin (1960) are synonymous with *S. aureus*; *M. cryophilus* is reported in *Bergey's Manual* as not producing acid from glucose and *M. saccharolyticus* is not listed there, nor is it mentioned by Kocur and Martinec (1962).]

The working hypothesis we propose, therefore, is that cocci can be divided into two subgroups on the basis of DNA per cent GC values, and that these subgroups coincide with the two taxometric subgroups. We have, therefore, proceeded to test the hypothesis by examining the DNA base ratios of organisms included in our taxometric studies and representative of both subgroups.

TABLE 1. *Per cent GC values reported in the literature for DNA from Staphylococcus and Micrococcus strains*

Organism	Per cent GC	Reference*
S. aureus SA-B	40.0	1
S. aureus 209P	37.7	1
S. aureus	34.7	1
M. pyogenes Oxford	30.7	1
M. pyogenes 1161	30.9	1
M. pyogenes 1149	31.2	1
M. pyogenes 145	31.4	1
M. pyogenes m 320	32.0	1
S. epidermidis	35.1	1
M. asaccharolyticus	34.1	1
M. cryophilus	38–40	2
M. halodenitrificans	64–66	2
M. lysodeikticus	71.9	1
Sarcina flava	68–70	2
Sarcina lutea	74.2	1
Sarcina lutea	72.0	1
Sarcina lutea	63.9	1

* Reference 1 = Belozersky and Spirin (1960); 2 = Marmur, Falkow, and Mandel (1963).

MATERIALS AND METHODS

Organisms. The following criteria were observed in choosing the strains to be examined for DNA base composition: (i) only strains deposited in a recognized collection would be used; (ii) both taxometric subgroups should be represented, and in approximate proportion to the number of strains included in them (however, the *Staphylococcus* subgroup comprised 37 strains of which only 11 were deposited ones, whereas the *Micrococcus* subgroup comprised 12 strains of which 10 were deposited ones); and (iii) all the names in the nomenclature of Shaw et al. (1951) should be represented. The organisms chosen are listed in Table 2 and comprise eight *Staphylococcus* strains and five *Micrococcus* strains.

Cultivation. Organisms were grown in nutrient broth at 37 C in aerated flasks. Optical density readings (λ = 560) were made at hourly intervals and plotted on semilogarithmic paper. Growth was interrupted as soon as a variation in the inclination of the growth curve was observed, to obtain cells at the end of exponential growth.

Lysis of the organisms and DNA preparation. S. afermentans and *S. roseus* strains were sensitive to lysozyme and were simply collected by centrifugation and their DNA was extracted as described by Marmur (1961). The other strains were either insensitive or poorly lysed by lysozyme, but it was possible to induce spheroplast formation by addition of 100 international units (IU)/ml of penicillin G. Spheroplasts, collected by centrifugation, were lysed with sodium lauryl sulfate, and again DNA extracted as described by Marmur (1961). Final fibrous DNA precipitates were dissolved in SSC (0.15 M NaCl + 0.015 M sodium citrate) or in 0.1 × SSC.

Determination of per cent GC. The same experimental procedure that we previously employed (Frontali, Hill, and Silvestri, 1965) was followed for determining the "melting temperatures," Tm, of the DNA preparations, from which the per cent GC can be calculated (Marmur and Doty, 1961). Determinations of *S. roseus* and *S. afermentans* DNA were carried out in 0.1 × SSC as their Tm values in SSC resulted at ca. 100 C and could not, therefore, be determined accurately in this solvent. To enable calculation of per cent GC from Tm in 0.1 × SSC, determinations of *Escherichia coli* K-12 (wild type) and *S. aureus* (NCTC 4136)

were made in both solvents. The linear dependence of Tm with the logarithm of the specific conductance of the solvent is well documented (T'so, Helmkamp, and Sanders, 1962; Frontali et al., 1965). The two straight lines joining the Tm values of *E. coli* and *S. aureus* in both solvents are parallel (Fig. 1) and, thus, by adding 15.4 C to the Tm in 0.1 × SSC, the expected Tm in SSC could be obtained. The per cent GC was calculated from Tm in SSC (actual or extrapolated) according to the relation of Marmur and Doty (1961): Tm = 69.3 + 0.41 (GC).

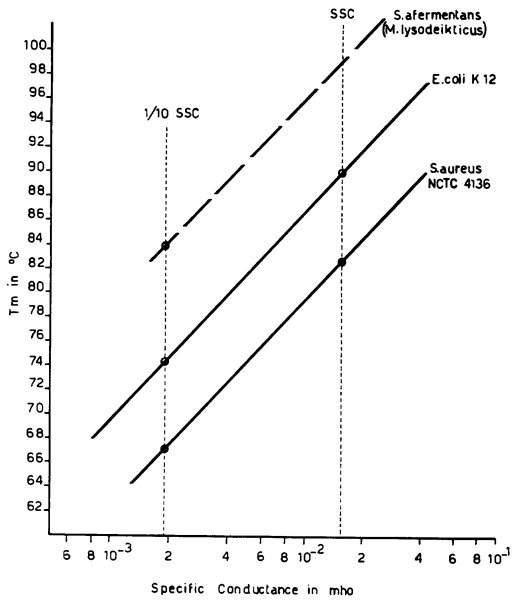

FIG. 1. *Tm values of two DNA preparations in both 0.1 × SSC and SSC, plotted against the specific conductance of these solvents (vertical dotted lines). The lines joining the two Escherichia coli Tm values and the two Staphylococcus aureus NCTC 4136 Tm values are very nearly parallel, thus permitting the addition of 15.4 C to a Tm value in 0.1 × SSC to extrapolate the expected Tm in SSC.*

RESULTS

The only common strain in our sample and in previous investigations (Table I) was strain NCTC 2665. The per cent GC of strain NCTC 2665, known as *S. afermentans* or *M. lysodeikticus*, evaluated from the extrapolated Tm was 72.8; this was in agreement with the previous chemical determination reported by Belozersky and Spirin (1960), who obtained a value of 71.9% GC.

Tm values, together with per cent GC values, are listed in Table 3. *S. aureus*, *S. saprophyticus*, and *S. lactis* strains had per cent GC values in the range 30.8 to 36.5. Data for *S. aureus* are more homogeneous (31.2 to 32.8% GC), and are in agreement with those of Table 1. *S. lactis* is evidently less homogeneous. Strain 31 (NCTC 7564), in particular, had a DNA base composition of 69% GC, much nearer to the values found for the *S. roseus* and *S. afermentans* strains, thus confirming the taxometric finding which places this strain in the genus *Micrococcus*. This strain was scored positive for acid production from glucose, thus conforming with the definition of Shaw et al. (1951) for *S. lactis*, under which name it was received. Strains received as *S. roseus* or *S. afermentans* all had high per cent GC values (72.8 to 75%).

TABLE 2. *Species of Micrococcaceae used in this study*

Strain no.*	Name†	NCTC no.‡	Other no. or name‡
1	*Staphylococcus aureus*	4136	
2	*S. aureus*	4163	
3	*S. aureus*	6571	ATCC 9144, Oxford H
4	*S. aureus*	8532	ATCC 12600
21	*S. saprophyticus*	7292	NCIB 8711
23	*S. saprophyticus*	7612	
29	*S. lactis*	189	
31	*S. lactis*	7564	
32	*S. lactis*	7944	
38	*S. roseus*	7511	
39	*S. roseus*	7512	
46	*S. afermentans*	2665	ATCC 4698, NCIB 9278, *M. lysodeikticus*
48	*S. afermentans*	7563	

* As in Hill (1959) and Hill et al. (1965).

† According to the nomenclature of Shaw et al. (1951).

‡ NCTC = National Collection of Type Cultures; ATCC = American Type Culture Collection; NCIB = National Collection of Industrial Bacteria.

TABLE 3. *Per cent GC values determined in this study for DNA from Staphylococcus and Micrococcus strains*

Strain no.	Name	Taxometric position[a]	Method of lysis[b]	Tm (C)		Per cent GC[d]
				0.1 × SSC	SSC[c]	
1	*S. aureus* NCTC 4136	A	P	67.3	82.7	32.8
2	*S. aureus* NCTC 4163	A	P	—	82.1	31.2
3	*S. aureus* NCTC 6571	A	P	—	82.4	32.0
4	*S. aureus* NCTC 8532	A	P	—	82.1	31.2
21	*S. saprophyticus* NCTC 7292	A	P	—	82.25	31.6
23	*S. saprophyticus* NCTC 7612	A	P	—	81.9	30.8
29	*S. lactis* NCTC 189	A	P	—	82.4	32.0
31	*S. lactis* NCTC 7564	B	P	82.25	(97.65)	69.0
32	*S. lactis* NCTC 7944	A	P	—	84.25	36.5
38	*S. roseus* NCTC 7511	B	L	84.8	(100.3)	75.0
39	*S. roseus* NCTC 7512	B	L	83.8	(99.2)	72.8
46	*S. afermentans* NCTC 2665	B	L	83.8	(99.2)	72.8
48	*S. afermentans* NCTC 7563	B	L	83.8	(99.2)	72.8

[a] A = *aureus-saprophyticus-lactis* group; B = *roseus-afermentans* group.
[b] P = penicillin; L = lysozyme.
[c] Values in parentheses are extrapolated ones.
[d] Tm (in SSC) = 69.3 + 0.41 (GC).

DISCUSSION

One of the controversial aspects of taxometrics, much discussed in recent years (see, for example, Heywood and McNeill, 1964), concerns the "reality" of groups formed in such analyses. Though much of the controversy may be due simply to different semantic meanings attached to the term "reality," this type of discussion contributes to the progress of taxonomy. As we have said elsewhere, any classification, whether it be phenetic, traditional, or other, ought always to be regarded as a hypothetical model to be tested experimentally (Silvestri and Hill, 1964).

In the present work, it was found that the hypothesis, derived from taxometric analyses, of a division of gram-positive, catalase-positive cocci into two main subgroups is confirmed by the study of DNA base compositions. The fact that those strains with low per cent GC values belong to one of the taxometric subgroups and that those with high per cent GC values belong to the other subgroup can not be due to chance. Particularly important is the behavior of strain 31.

From the present study, the following methodological principles can be proposed: (i) phena or groups based on overall similarity which satisfy criteria for stability (which means, at present, groups repeatedly formed with different taxometric methods) can be confirmed by use of experimental criteria independent of taxometric criteria, (ii) conversely, whenever members of a monothetic group, i.e., a group defined by the obligatory possession of one or a few characters (e.g., the "group" gram-positive, catalase-positive cocci), show heterogeneity in DNA base compositions, it may be expected that the group would become split in a taxometric analysis, yielding subgroups which coincide with the base ratios.

Bacterial taxonomy would be greatly simplified if only groups found to be stable and homogeneous in the sense used throughout this paper were to be accepted as "really" autonomous taxonomic entities. Acceptance of only such groups would lead to a considerable reduction in the number of taxonomic entities. It would favor a process of "lumping" together many of the species now recognized, which are excessively numerous as a consequence of traditional and, above all, monothetic "splitting."

Cowan and Steel (1964) recently drew attention to the difficulty encountered in attributing some strains of *S. saprophyticus* and *S. lactis* to one or other of the two genera *Staphylococcus* and *Micrococcus* on the basis of a single character. Attempts to find a single character which, independently, can separate two groups such as staphylococci and micrococci are, we think, bound to fail. Moreover, it is probable that the staphylococci-micrococci are not a particular, but rather a common, case. Division of such systems can only be achieved by considering many phenetic characters and by estimating average DNA base compositions (in cases, such as the present, in which these are distinguishable).

The empirical criterion used here for the stability of the phena is undoubtedly not the best, in that it calls for the use of different taxometric analyses and that it can be criticized for its subjectivity. Better criteria can be evolved (Silvestri et al., 1963; Rogers and Fleming, 1964), and the future development of taxometrics will indeed depend on the introduction of such criteria.

The finding that the cocci studied here can be divided into two subgroups with widely different DNA base compositions suggests that the group as a whole is phylogenetically heterogeneous (Sueoka, 1964), and that the common characters (gram-positive, catalase-positive, coccus morphology) are due to evolutionary convergence. However, Gause et al. (1964) reported that, after ultraviolet irradiation of a staphylococcus with a DNA base composition of 32.4% GC, several small-colony mutants were isolated whose DNA base compositions varied between 69.2 and 71.0% GC. It is remarkable that the per cent GC values for these mutants are typical of the micrococci. The description given of the mutants is not sufficient to establish whether they could indeed be identified as such. Before drawing conclusions concerning the phylogenetic independence of the genera *Staphylococcus* and *Micrococcus*, it will be necessary to establish in detail the nature of the mutants of Gause et al. (1964).

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