THE RELATIONSHIPS BETWEEN CERTAIN MEMBERS
OF THE STAPHYLOCOCCUS-MICROCOCCUS GROUP
AS SHOWN BY THEIR CELL WALL COMPOSITION

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Many attempts have been made to devise a satisfactory classification of the Gram-positive, catalase-positive cocci, but there is as yet no general agreement as to how the group can best be subdivided or what the subdivisions shall be called. Wilson and Miles (1955), for example, would recognize Staphylococcus, Micrococcus, Sarcina and Rhodococcus as separate genera, while Breed et al. (1948) defined 3 genera, Micrococcus, Sarcina and Gaffkya. Shaw, Stitt and Cowan (1951) on the other hand, regarded the whole group as being of one genus, Staphylococcus, and rejected the name Micrococcus as invalid.

In the course of an investigation into the chemical composition of the cell wall in Gram-positive bacteria, (Cummins and Harris, 1955, 1956) the results obtained suggested that cell wall composition was a useful taxonomic character. It seemed of interest therefore to analyse cell wall fractions from a number of strains of the staphylococcus-micrococcus group to see whether the results would be of any value in the classification of the group as a whole.

MATERIALS AND METHODS

Strains of Bacteria.

Of the 24 strains examined, 7 were isolated from nasal swabs in The London Hospital by Dr. F. C. O. Valentine. These comprised 3 strains of Staphylococcus aureus, 3 of S. albus, and 1 of S. citreus. These 7 strains were selected in the first place on the basis of colonial pigmentation; subsequent testing for coagulase production confirmed that the S. aureus strains were coagulase positive while the other four were negative. All other strains were obtained from the National Collection of Type Cultures.

Table 1. Principal components in the cell walls of 24 strains of the staphylococcus-micrococcus group.

	Galactose	Glucose	Mannose	Unknown carbo- hydrate material	Glucosamine	Galactosamine	Unknown 'Hexosamine'	Alanine	Glutamic acid	Lysine	Glycine	Diaminopimelic acid
GROUP I.												
<u>Staphylococcus aureus</u> (3 strains)	-	-	-	-	+	-	+	+++	+++	+++	+++	
<u>Staphylococcus aureus</u> NCTC 7415	-	-	-	-	+	-	+	+++	+++	+++	+++	
<u>Staphylococcus albus</u> (3 strains)	-	-	-	-	+	-	+	+++	+++	+++	+++	
<u>Staphylococcus albus</u> NCTC 7944	-	-	-	-	+	+	+	+++	+++	+++	+++	
<u>Staphylococcus saprophyticus</u> NCTC 7292	-	-	-	-	+	-	+	+++	+++	+++	+++	
<u>Staphylococcus saprophyticus</u> NCTC 7291	-	Tr	-	-	+	-	+	+++	+++	+++	+++	
<u>Sarcina ureae</u> NCTC 4819	-	-	-	-	+	-	+	+++	+++	+++	+++	
<u>Staphylococcus citreus</u> (1 strain)	-	+	+	-	+	-	+	+++	+++	+++	+++	
<u>Sarcina lutea</u> NCTC 611	-	++	-	-	+	-	+	+++	+++	+++	+++	
<u>Micrococcus luteus</u> NCTC 8512 (ATCC 398)	-	++	-	-	+	-	+	+++	+++	+++	+++	
<u>Micrococcus lysodeikticus</u> NCTC 2665	-	++	-	-	+	-	+	+++	+++	+++	+++	

<u>GROUP II.</u>												
<u>Micrococcus conglomeratus</u> (NCTC 2677)	++	+	-	-	+	Tr	+	+++	+++	+++		
<u>Staphylococcus lactis</u> NCTC 7564	++	+	-	-	+	Tr	+	+++	+++	+++		
<u>Micrococcus roseus</u> NCTC 7520	++	Tr	-	-	+	Tr	+	+++	+++	+++		
<u>Aerococcus viridans</u> NCTC 8251	-	+	+	-	+	+	+	+++	+++	+++		
<u>Aerococcus viridans</u> NCTC 7593	-	+	-	-	+	+	+	+++	+++	+++		
<u>Aerococcus viridans</u> NCTC 7592	+	+	-	-	+	+	+	+++	+++	+++		
<u>Aerococcus viridans</u> NCTC 7764	+	+	Tr	-	+	+	+	+++	+++	+++		
<u>GROUP III.</u>												
<u>Micrococcus varians</u> NCTC 7281	++	-	-	++	+	-	+	+++	+++		++	++
<u>Micrococcus citreus</u> NCTC 7731	++	-	-	++	+	-	+	+++	+++		++	++

Cultural Methods.

The organisms were grown on nutrient agar at 37°C for 24-48 hours, or, in the case of some more slowly growing strains such as Micrococcus conglomeratus (NCTC 2677) in nutrient broth in conical flasks, the volume of medium being adjusted to give good aeration. After harvesting, the suspensions were washed twice in distilled water and preserved in 0.5 per cent formol saline if not disintegrated at once.

Preparation and Analysis of Cell Wall Fractions.

The method of preparing cell wall fractions, and the chromatographic techniques used have been described in detail elsewhere (Cummins and Harris, 1956). Briefly, the organisms were disintegrated by shaking with glass beads (balotini, 0.18-0.2 mm diam.), and the insoluble (cell wall) fraction separated by centrifugation and purified by digestion with trypsin and ribonuclease, followed by pepsin. Acid hydrolysates (100°C) of this material were examined by two-dimensional paper chromatography and the amino acids, sugars, and amino sugars identified. The amount of each component present was recorded as +++, ++, +, $\frac{+}{-}$, Tr, or - depending on the size and intensity of the spots.

RESULTS

The results obtained in the 24 different strains examined are given in Table I. The findings in some of these organisms have already been reported (Cummins and Harris, 1956), but are included here for the sake of completeness. For each organism, data are presented concerning the occurrence of three separate groups of substances in the cell wall hydrolysates; amino acids, amino sugars, and sugars. For simplicity only the major components present are recorded in the case of the amino acids. In every instance these three or four major amino acid components accounted for a very high proportion of the total amino acids present in the preparations and they gave rise to clear cut and readily recognisable patterns. Small amounts of a number of other amino acids were also to be found in some of the preparations. The significance of these minor components is still uncertain and the problem has been discussed elsewhere (Cummins and Harris, 1956). It is possible that in some

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cases they arise because the cell wall preparation had not been completely freed from extraneous proteins. In none of these preparations was any ribose identified, which indicates that they had been effectively freed from nucleic acid.

In our previous survey of the cell wall components of different types of Gram-positive bacteria it appeared that differentiation between large groups of organisms at the generic level was provided most clearly by the pattern of the major amino acid components. The pattern of the sugar and amino sugar distribution, on the other hand, appeared more to reflect differences between species or strains, although the occurrence of certain sugars such as arabinose in the corynebacteria and rhamnose in the streptococci probably has a broader significance.

The amino acid pattern was therefore expected in the present survey of Gram-positive cocci to reveal any large taxonomic divisions which might be present. In fact, it was found that the bacteria studied fell quite sharply into three main groups with respect to the pattern of the major amino acid components of the cell walls.

Group I.

Characteristic cell wall amino acids; alanine, glutamic acid, lysine, and glycine.

This is the largest group in the present series (15 out of 24 strains) and includes representatives of the species normally parasitic on man. Within the group the findings seem to suggest a division between the first 11 strains and the 4 remaining ones, in that the latter contain appreciably more sugar in their cell walls. Another possible point of distinction, omitted from Table I, is that the cell wall preparations from Staphylococcus aureus, S. albus, and S. saprophyticus contain small but appreciable amounts of serine whereas this is apparently absent from preparations of the other strains in Group I (see Cummins and Harris, 1956).

The results in the case of Micrococcus luteus (ATCC 398) are of some interest, since this strain has been proposed as the type species of the genus Micrococcus (Breed, 1952). The difference in cell wall composition between this strain and the strains of S. albus and S. aureus is in the presence or absence of hexose, and to judge by the results in the other genera (e.g., Streptococcus, Lactobacillus, and Corynebacterium) this would point to a difference in species but not

in genus, since they have the same characteristic cell wall amino acids. The results of cell wall analysis, therefore, so far as they go in this small series, do not support the separation of Micrococcus and Staphylococcus as distinct genera.

Group II.

Characteristic cell wall amino acids; alanine, glutamic acid and lysine; glycine absent or present only in traces.

The first 3 strains in this group (2677, 7564, and 7520) have cell wall compositions closely resembling one another, with considerably less galactosamine than glucosamine. The 4 strains of Aerococcus viridans, on the other hand, show considerable variation in the type and amount of hexose present, but all have galactosamine in approximately equal amounts in their cell walls.

Group III.

Characteristic amino acids; alanine, glutamic acid, α , ϵ - diaminopimelic acid, and glycine.

These 2 strains are unusual in that diaminopimelic acid has not been previously reported in Gram-positive cocci, and also because of the occurrence in their cell walls of an unidentified sugar component. This material has not been observed in any of the other strains or indeed in any other organism we have examined. It moves very slowly in phenol/water ($R_f \bar{c}$ = galacturonic acid), but more rapidly in lutidine ($R_f \bar{c}$ = galactose). It reacts with aniline hydrogen phthalate in a similar manner to the hexoses, but it fails to react with naphtho-resorcinol and so presumably is not a uronic acid. It does not correspond in chromatographic behaviour to any known monosaccharide and may well be an oligosaccharide "core" relatively resistant to hydrolysis. It is of interest that it should have been encountered only in the two organisms with a cell wall amino acid pattern different from that found so far in any other Gram-positive cocci.

Table II. Comparison of classification of 17 strains of staphylococci (Shaw, Stitt and Cowan, 1951) with the grouping of the same strains on cell wall composition.

Species name Shaw, Stitt and Cowan 1951	NCTC number	Group by cell wall composition (see Table I)	Other names for strains
<u>Staphylococcus aureus</u>	7415	I	<u>Staphylococcus epidermidis</u>
<u>Staphylococcus saprophyticus</u>	7292	I	<u>Staphylococcus epidermidis</u>
	7291	I	<u>Staphylococcus albus</u>
<u>Staphylococcus lactis</u>	7944	I	<u>Staphylococcus albus</u>
	2677	II	<u>Micrococcus conglomeratus</u>
	7564	II	
	7281	III	<u>Micrococcus varians</u>
<u>Staphylococcus roseus</u>	7520	II	<u>Micrococcus roseus</u>
<u>Staphylococcus afermentans</u>	7731	III	<u>Micrococcus citreus</u>
	4819	I	<u>Sarcina ureae</u>
	2665	I	<u>Micrococcus lysodeikticus</u>
	8512	I	<u>Micrococcus luteus</u>
	611	I	<u>Sarcina lutea</u>
α -group	8251	II	
	7593	II	<u>Aerococcus viridans</u>
	7592	II	(Williams, Hirsch and
	7764	II	Cowan, 1953)

DISCUSSION

The results reported here indicate that it is possible to divide the Gram-positive catalase-positive cocci into at least 3 well-defined groups on the basis of cell wall composition, but these do not correspond to the subdivisions suggested in any recent classification of these organisms. We were able to include in the 24 strains examined, 13 representatives of the 5 species of Staphylococcus proposed by Shaw, Stitt and Cowan (1951) and also 4 strains of their α -group, later named Aerococcus viridans (Williams, Hirsch and Cowan, 1953). The results of cell wall analysis on these 17 strains are shown in Table II, and it is evident that there is no agreement between the two methods of subdivision. This is particularly noticeable in the case of the strains labelled Staphylococcus lactis, at least one of which falls into each of the 3 cell wall groups.

We do not wish in this paper to suggest a new classification of the staphylococcus-micrococcus group, nor to criticise (or support) any of the existing ones, but to bring to the notice of those interested in taxonomic problems a bacterial character which may be of value in this field. The use of cell wall composition as a taxonomic character in Gram-positive bacteria has been discussed elsewhere (Cummins and Harris, 1956). The arguments there put forward in its favour are, briefly, that the composition of the cell wall seems to be quite unaffected by growth conditions, that the components present can be identified by comparison with artificial standards and that where differences in the pattern of amino acid components occur, these differences are large and clear-cut. No character, however, can have such overriding significance that all others must give way to it, and the final taxonomic value of cell wall composition has yet to be determined.

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