Deoxyribonucleic Acid Base Composition of *Staphylococcus epidermidis* and its Phages

By J. VERHOEF, A. J. HOFF,
BERNARDA HOLTRIGTER AND A. C. M. VAN DER DRIFT

Laboratory of Microbiology, State University, Catharijnesingel 59, Utrecht,
Laboratory of Physiological Chemistry, State University, Leiden and
Medical Biological Laboratory TNO, Rijswijk, The Netherlands

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SUMMARY

The base composition of DNA preparations from 16 strains of staphylococci, belonging to different subgroups, and from 10 staphylococcal phages were determined from the denaturation temperature (T_m) and from the buoyant density. The GC (guanine + cytosine) content of the DNA of the strains ranged from 29.3 to 33.9 % (calculated from T_m) and from 28.5 to 34.0 % (calculated from the buoyant density). No significant difference was observed between the GC content of the DNA of the strains of *Staphylococcus aureus* and *S. epidermidis* nor between its various biotypes. The mole fraction GC of staphylococcal phage DNA (31.4 to 34.6 % GC) is somewhat higher than the GC content of the DNA of the strains.

INTRODUCTION

The taxonomy of staphylococci has long been a matter of considerable difficulty and was subject to various misunderstandings. The classification of the Micrococcaceae by Baird-Parker (1963) seems to be the most adequate. Based on a number of biochemical tests, Baird-Parker recognized six subgroups of staphylococci. Subgroup I includes *Staphylococcus aureus*, subgroups II to VI comprise the coagulase-negative staphylococci (*S. epidermidis*). The latter subgroups are also indicated as biotypes 1 to 5 (Baird-Parker, 1965*b*).

A major characteristic in the taxonomy of bacteria is the deoxyribonucleic acid base composition (Belozerky & Spirin, 1960). It was pointed out by Silvestri & Hill (1965) that aerobic Gram-positive and catalase-positive cocci can be divided into two groups based on the guanine + cytosine (GC) content of their DNA's. The first group, with a low % GC in DNA (30 to 35 %) corresponds to the genus *Staphylococcus* and the second group with a high GC percentage (about 70 %) corresponds to the genus *Micrococcus*. These conclusions were confirmed by Rosypal, Rosypalova & Horřejs (1966) and by Garrity, Detrick & Kennedy (1969). Not much attention was given to the GC content of *Staphylococcus epidermidis*, and possible differences within the group of staphylococci were not considered.

As staphylococci of the various biochemical subgroups also differ in habitat and in susceptibility to specific phages (Baird-Parker, 1965*a*; Holt, 1969; Verhoef, 1970), it seemed of interest to study the range of GC content of the DNA of strains belonging to various subgroups of *Staphylococcus epidermidis* and of *S. epidermidis* phage-DNA in comparison with the GC content of the *S. aureus*.

METHODS

Cultures. Coagulase-negative staphylococci were collected from staff, patients and air in a hospital, and from second year students. The coagulase-positive staphylococci used are propagating strains for the International Set of Typing-phages for *Staphylococcus aureus* and were received from the National Institute of Public Health, Bilthoven, The Netherlands. The organisms used were classified into subgroups or biotypes as described previously (Verhoef, van Boven & Winkler, 1971*a*) and are listed in Table 1.

Phages. Phages from *Staphylococcus epidermidis* biotype 1, 3, 4 and 5 were isolated and characterized as described elsewhere (Verhoef *et al.* 1971*a, b*). *Staphylococcus aureus* phages, belonging to the International Set of Typing-phages, were received from the National Institute of Public Health, Bilthoven, The Netherlands. The phages used in this investigation and their origin are listed in Table 2.

Phages were grown in Nutrient Broth (Difco), with 0.004 M-CaCl₂. Two litres of broth were inoculated with 10⁸ to 10⁹ plaque-forming units (p.f.u.) of phage and with 50 ml. of an 18 h. culture of the propagating strain. After 18 h. incubation at 37° the titre of the lysate was about 10⁹ p.f.u./ml. The phages were purified by two cycles of differential centrifugation and subsequent banding in a CsCl gradient.

Extraction of DNA from bacteria and phages. DNA was extracted from bacteria by the method of Marmur (1961). Cells were lysed according to procedures described by Perry & Slade (1964). The DNA preparation was diluted in a 0.04 × SSC solution (SSC contains 0.15 M-NaCl and 0.015 M-Na₃citrate).

Phage DNA was isolated by phenol extraction as described previously by Lee & Davidson (1968) and dialysed against 0.04 × SSC.

Determination of the mole fraction GC base pairs. The GC percentages were calculated from the density and from the melting-temperature (*T_m*) of the DNA.

DNA densities were determined by density gradient sedimentation in a Beckman Model E analytical ultracentrifuge, equipped with a photoelectrical scanning attachment and temperature regulator. All runs were performed at 44,770 rev./min. during 20 h. at a constant temperature of 20°. The density profile was obtained by scanning at 264 nm. The density was calculated in the usual manner.

DNA of *Micrococcus leisodeikticus* was used as a marker; GC percentages were calculated according to De Ley (1970). Melting curves were measured by heating the DNA in a 1 cm. path length quartz cuvet in jacketed housing, mounted in the cell compartment of a Beckman DK2 spectrophotometer. Sample temperatures were measured with a glass-enclosed thermistor, dipping into the top of the sample through a glass stopper. Sample temperatures were changed at a rate of 0.2°/min. by circulating water from a Colara water bath, equipped with a motor-driven temperature regulator through the jacketed housing. This rate is sufficiently slow to permit the DNA strands to unwind without lagging (Hoff & Blok, 1970). The heating produced some single-strands breaks in the DNA (Hoff, 1971), but did not affect the melting curve.

The absorbance at 259 nm. was measured by operating the spectrophotometer in the transmission mode and using the logarithmic slidewire as a retransmitting potentiometer. In this way the absorbance scale could be expanded while maintaining the stability and the low noise characteristics of the split-beam system. Melting curves of DNA samples of concentration as low as 5 µg./ml. could be accurately measured. Signals corresponding to absorbance and temperature were recorded simultaneously on a Mosely X-Y recorder. The accuracy of the absolute temperature is better than 0.1°, that of temperature differences

about 0.01° . From the T_m values GC percentages were calculated using the general formula of Owen, Hill & Lapage (1969), which relates the GC percentage, T_m and the ionic strength of the solution. The latter was checked by conductivity measurements.

RESULTS AND DISCUSSION

The mole fraction guanine + cytosine of the DNA of the staphylococci studied are summarized in Table 1. There is only a slight difference between the GC percentages obtained by the determination of the denaturation temperature (T_m) and those obtained by the determination of the buoyant density. This difference (about 2 % GC) is probably not significant. The reproducibility of the T_m is better than 0.2° , corresponding to 0.5 % GC. The density determinations are somewhat less accurate. Due to the uncertainty in the density of the marker DNA, which is known to only 3 decimal places, the systematic error in the absolute GC content may be as large as 1 % GC. On this are superimposed random errors, mainly due to the uncertainty in the position of the density peaks, which sum to about 1.5 % GC.

Table 1. % GC values of staphylococcal DNA

Strain number	Origin of strain	Sub-group	Susceptibility to phage	Buoyant density	% GC determined by buoyant density	T_m in $0.04 \times \text{SSC}$	% GC determined by T_m
82	(1)	I	++	1.6927	32.3	58.2	32.4
83 A	(1)	I	++	1.6905	30.0	59.0	33.9
407	Skin	II	+	1.6907	30.2	.	.
382	Skin	II	+	1.6907	30.2	57.9	31.8
1505	Air	II	+	1.6913	30.8	.	.
2	Cow	III	—	1.6943	34.0	.	.
13	Cow	III	—	1.6905	30.0	.	.
1555	Nose	IV	—	1.6921	31.7	58.1	32.2
P I	Patient's skin	IV	+	1.6900	29.5	56.6	29.3
1450	Air	IV	—	1.6917	31.3	.	.
1521	Air	IV	—	1.6921	31.7	.	.
1561	Nose	V	—	.	.	57.0	30.0
1635	Clinical material	V	—	1.6890	28.5	58.6	33.1
1520	Air	VI	—	1.6918	31.4	.	.
1522	Air	VI	—	1.6928	32.4	57.0	30.0
1631	Skin	VI	—	1.6920	31.6	.	.

(1) Propagating strain of the International Phage-typing Set of *Staphylococcus aureus*.

++ *S. aureus* typing phages.

+ *S. epidermidis* typing phages (Verhoef, van Boven & Winkler, 1971 c).

— Not susceptible to any of the phages mentioned above.

According to De Ley (1970) the confidence limits derived from the linear regression lines are approximately 4.3 % and 3.7 to 4.1 % GC for values calculated from the buoyant density and T_m respectively. The differences in GC content in our material fall well within these limits. As can be seen from Table 1 the GC content of the DNAs of the strains tested varies within the range of 29.3 to 33.9 % GC (determined from the T_m) or within the range 28.5 to 34.0 % GC (determined from the buoyant density). There is only a slight difference between *Staphylococcus aureus* and *S. epidermidis* DNA (32.4 and 33.9 respectively 29.3 to 33.1; determined from the T_m). These results are in accordance with the values for staphylococcal DNA found by other authors (Silvestri & Hill, 1965; Auletta & Kennedy, 1966; Boháček, Kocur & Martinec, 1967; Klesius & Schuhradt, 1968; Garrity *et al.* 1969). Furthermore our

results show that various biotypes and phage-groups within the *Staphylococcus* group do not significantly differ in their GC content. Consequently the GC content can not be used to differentiate between *S. aureus* and *S. epidermidis*. In practical bacteriology one still has to rely on the fermentation of mannitol under anaerobic conditions, and on the production of coagulase as the differential characteristics (see Baird-Parker, 1963).

Table 2. *Mole fraction GC of staphylococcus phage DNA*

Phage number	Subgroup of lysogenic strains from which the phage originated	Buoyant density	% GC
*6	I	1·6918	31·4
*54	I	1·6923	31·9
*55	I	1·6947	34·4
82	II	1·6949	34·6
459	II	1·6947	34·4
A1	IV	1·6940	33·6
1499	IV	1·6943	34·0
157	V	1·6947	34·4
1548	VI	1·6931	32·7
1427	VI	1·6938	33·4

* Phages belonging to the International Phage-typing Set of *Staphylococcus aureus*.

The mole fraction GC of staphylococcus phage DNA ranged from 31·4 to 34·6 % GC (Table 2). They are somewhat higher than those of the staphylococcal DNA studied. Similarly there is no significant difference in GC content of DNA of phages derived from different staphylococcal subgroups.

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