Deoxyribonucleic Acid Base Composition of Serotype Strains of *Staphylococcus aureus*

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SUMMARY

The percentage guanine + cytosine (GC) content of the DNA of 19 serotype strains of *Staphylococcus aureus* determined from melting temperatures (T_m) and the E_{260}/E_{280} ratio at pH 3 ranged from 30.2 to 35 % GC, average 32.5 % GC.

INTRODUCTION

DNA base composition has become a criterion for differentiation between staphylococci and micrococci (see for example Silvestri & Hill, 1965; Auletta & Kennedy, 1966). Therefore it is important to know the molar percentage of guanine plus cytosine in the total base content of the DNA (% GC) of those staphylococci which are used as working types, as serotyping strains and for bio-assays.

We report here the DNA base composition of the strains of *Staphylococcus aureus* recommended for serological typing by Oeding (1952, 1957) and Haukenes (1967).

METHODS

All the 19 strains of *Staphylococcus aureus* investigated (Table 1) were supplied by Dr P. Oeding, Department of Microbiology, University of Bergen, Norway, except CCM885 which was obtained from the National Collection of Type Cultures.

Isolation of DNA. Lysis was carried out using lysostaphin by the method of Klesius & Schuhardt (1968), but the subsequent process of recommended isolation was modified. Bacteria grown on nutrient agar at 37° for 24 h. were suspended in tris-saline-citrate (TSC) buffer; 0.05 M-tri-(hydroxymethyl)methylamine (tris), 0.145 M-NaCl, 0.015 M-trisodium citrate; adjusted to pH 7.4 with HCl. After centrifugation, the bacteria were washed once with TSC and then resuspended in TSC, using 20 ml. for each 3 g. wet weight. Lysis was induced by adding 100 units lysostaphin (Mead Johnson, Research Centre, Evansville, Indiana, U.S.A.) and incubating at 37° for 30 to 60 min. 30 ml. of saline-EDTA solution (0.15 M-NaCl, 0.1 M-EDTA, pH 8) and 5 ml. of a 20 % (w/v) solution of sodium lauryl sulphate were added. The lysate was left in a stoppered Erlenmeyer flask in a water bath at 60° for 15 min., cooled, and an equal volume of chloroform-isoamyl alcohol (24:1, v/v) was added. The mixture was shaken for 20 min. and centrifuged to separate the upper aqueous layer from which the DNA was precipitated by addition of 1.5 vol. of redistilled ethanol.

The precipitated DNA was dissolved in tenfold diluted standard saline-citrate (SSC) buffer: 0.15 M-NaCl, 0.015 M- Na_3 citrate and incubated with 50 $\mu\text{g./ml.}$ RNase (Calbiochem, Medford Street, Los Angeles, California, U.S.A.) at 37° for 24 h. Deproteinization was

completed by repeated shaking with chloroform-isoamyl alcohol. The DNA was precipitated again and, after redissolving, the turbid solution was cleared by centrifuging at 70,000 g for 45 min.

Table 1. *List of strains used*

<i>Staphylococcus aureus</i> CCM no.	Other numbers
1486, 2317, 2323, 2326, 2327, 2328, 2330, 2331, 2339, 2350, 2457, 2458, 2459, 2460, 2461 885	P. OEDING no. 263; 1503; 2253; 28; 365; 3647; F21; 17A; 3189, 2095; 1015; 670; 6376; 5687 and 830 ATCC 12600; NCTC 8532; CIP 65.8; PZH 727/55; strain s33 R4; COWAN III
2351	ATCC 10832; NCTC 7121; PZH 590/54; strain WOOD 46
2352	ATCC 12598; NCTC 8530; CIP 65.6; PZH 725/55; COWAN I
2353	ATCC 12599; NCTC 8531; PZH 726/55; COWAN II

Abbreviations: ATCC = American Type Culture Collection, Rockville, Maryland, U.S.A.; CCM = Czechoslovak Collection of Micro-organisms, Brno, Czechoslovakia; CIP = Collection of Institut Pasteur, Paris; NCTC = National Collection of Type Cultures, London; PZH = Collection of Hygiene Institute, Warsaw.

Table 2. *DNA base ratios (GC) of 19 serotypes of Staphylococcus aureus*

<i>Staphylococcus aureus</i> CCM no.	T_m *	% GC†	E_{260}/E_{280} ‡	% GC§
2457	64.6	30.2	1.580	33.0
2353	64.7	30.4	1.560	34.5
2317	65.0	31.1	1.580	33.0
2459	65.0	31.1	1.550	35.0
2351	65.0	31.1	1.600	31.7
2350	65.0	31.1	1.570	33.5
2331	65.0	31.1	1.580	33.0
2339	65.1	31.4	1.570	33.5
1486	65.2	31.6	1.565	34.0
2330	65.4	31.8	1.570	33.5
2323	65.4	31.8	1.600	31.7
2458	65.4	31.8	1.570	33.5
2460	65.4	31.8	1.575	33.2
2326	65.6	32.4	1.590	32.5
2327	65.6	32.4	1.570	33.5
2461	65.7	32.7	1.580	33.0
2352	65.7	32.7	1.560	34.5
885	65.7	32.7	1.550	35.0
2328	65.9	33.1	1.575	33.3

* T_m : denaturation temperatures obtained in PE buffer.

† % GC: calculated according to the equation $GC = (T_m - 51)/0.45$.

‡ E_{260}/E_{280} : extinction ratios at pH 3.0.

§ % GC: interpolated from Fredericq *et al.* (1961) non-linear graphical function.

The clear solution containing DNA was carefully poured off and after adding 0.1 vol. of a solution containing 3 M-sodium acetate and 0.001 M-EDTA, pH 7, the DNA was reprecipitated by gradual addition of ethanol (1 to 1.5 vol.). The DNA fibres were dissolved in phosphate-EDTA (PE) buffer: 0.01 M-sodium phosphate, 0.001 M-EDTA, pH 7. The protein content in the samples ranged from 0.5 to 3 % (Folin reaction) and polysaccharides were estimated in the range of 2 to 7 % (anthrone reaction; Roe & Dailey, 1966).

Determination of the base composition of DNA. This was determined by two different methods: from the T_m values measured in PE buffer, and from the extinction ratio E_{260}/E_{280} measured spectrophotometrically in 0.1 M-acetic acid (Fredericq, Oth & Fontaine,

Table 3. The biochemical characteristics of 19 serotype strains of *Staphylococcus aureus*

<i>Staphylococcus aureus</i> CCM no.	Lactose	Tween 80	Egg yolk	Fibrino- lysin	Haemo- lysin	Urease	Serological pattern*
2457	+ ³	+ ³	+ ³	+	—	+	c ₁
2353	+ ¹	+ ¹	+ ²	+	β	—	h ₂ /263-2
2317	+ ¹	+ ¹	+ ²	—	—	—	e/m/n
2459	+ ¹	+ ¹	—	—	αβ	+	c ₁
2351	+ ¹	+ ¹	+ ²	+	αβ	—	i ₁ i ₂
2350	+ ¹	+ ¹	+ ²	—	—	—	a ₄ /a ₅ /b ₁
2331	+ ¹	+ ²	+ ²	—	—	—	a ₅ /h ₁ /h ₂ /263-2
2339	+ ¹	+ ²	+ ²	—	—	—	NT
1486	+ ¹	+ ¹	+ ¹	+	α	—	a ₅ /k ₁ k ₂ /m/263-1/263-2
2330	+ ¹	+ ¹	+ ²	—	—	—	b ₁ /c ₁ /i ₁ i ₂ /m
2323	+ ¹	+ ²	+ ²	—	—	—	c ₁ /e/m/n
2458	+ ³	+ ³	+ ³	—	β	+	h ₁ /h ₂
2460	+ ¹	+ ¹	—	—	β	+	h ₂ /263-2
2326	—	+ ²	+ ²	—	—	—	e/n
2327	+ ¹	+ ¹	+ ²	—	—	—	b ¹ /k ¹
2461	+ ¹	+ ¹	—	+	α	+	a ₅
2352	+ ¹	—	—	—	—	—	h ₂ /k ₁ k ₂ /m/263-1/263-2
885	+ ¹	+ ¹	+ ³	—	αβ	+	a ₄ /a ₅ /b ₁ /c ₁ /n
2328	+ ¹	—	—	—	—	—	a ₄ /a ₅ /b ₁ /c ₁ /n

All strains studied were catalase and benzidine test-positive, produced free and/or bound coagulase, produced acid anaerobically and aerobically from glucose and mannitol, produced acid aerobically from maltose and galactose, produced acetoin and phosphatase, were methyl-red positive, reduced nitrate and nitrite, produced NH₃ from arginine, grew at 45° and in presence of 15 % NaCl.

None of the strains hydrolysed aesculin or starch, nor produced ornithine or lysine decarboxylases, nor phenylalanine deaminase, nor grew on Simmons citrate agar.

+¹, +², +³ = Positive reaction after 1, 2 and 3 days respectively; — = Negative reaction; NT = Not typable, control strain. * P. Oeding, personal communication.

1961). A detailed description of the methods used is given by Boháček, Kocur & Martinec (1967).

Morphological properties and biochemical characteristics of the strains were studied according to the methods used by Mortensen & Kocur (1967).

RESULTS

The GC contents of the DNA of the 19 strains of *Staphylococcus aureus* are given in Table 2. The GC contents show a narrow range: 30.2 to 33.1 % GC (average 31.7) from the *T_m* values measured in PE buffer, while a slightly wider range was found 31.7 to 35 % GC (average 33.4 % GC) from the extinction ratios. The differences between the values obtained by these two methods ranged in most strains from 0.1 to 2 % GC. Only three of the strains (CCM2457, 2353 and 2459) showed differences of 3 to 4 % GC. With the exception of strain CCM2323, higher values were obtained by the method of Fredericq *et al.* (1961). This fact has been already discussed in our previous work (Boháček, Kocur & Martinec, 1970).

The biochemical characteristics and serological patterns of 19 strains of *Staphylococcus aureus* are given in Table 3.

DISCUSSION

The isolation of DNA from strains of *Staphylococcus aureus* is difficult because the commonly used methods of enzymic and chemical lysis of bacterial cells are not very effective. The induction of osmotically fragile spheroplasts by adding penicillin to the growth medium is an exception, but this method has apparently been used for the purpose of isolating DNA by only Silvestri & Hill (1965). The lytic enzyme lysostaphin, discovered by Schindler & Schuhardt (1964), causes a rapid lysis of *S. aureus* cells and makes the isolation of DNA possible.

Our results are in agreement with the data obtained by Garrity, Detrick & Kennedy (1969) and Klesius & Schuhardt (1968), whose finding that lysostaphin was very effective in the isolation of DNA from staphylococci is confirmed. Two of our strains have been studied by other authors. For the strain CCM 885 (NCTC 8532), 31.2 % GC was reported by Silvestri & Hill (1965), in comparison with our finding of 32.7 % GC (from T_m). Garrity *et al.* (1969) reported 33.4 % GC for strain CCM 2351 compared with our estimate of 31.1 % GC (again from T_m). Differences of +1.5 and -2.3 % GC, respectively, slightly exceed the range of the maximal experimental error reported by Marmur & Doty (1962) which was 1 mole % GC. Theoretical motivation of discrepancies in the results of GC measurements performed in buffers of different ionic strength has been recently reported by Owen, Hill & Lapage (1969).

No correlation between GC content and a particular serological pattern or biochemical characteristics of *Staphylococcus aureus* strains was found.

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