Rapid Procedure for the Approximate Determination of the Deoxyribonucleic Acid Base Composition of Micrococci, Staphylococci, and Other Bacteria

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A simple and rapid procedure for the isolation of bacterial deoxyribonucleic acid is described. The deoxyribonucleic acid preparations are pure enough to determine the base composition by ultraviolet spectroscopy.

Currently the most reliable test for the separation of staphylococci and micrococci is either an analysis of their deoxyribonucleic acid (DNA) base composition or the determination of their cell wall components. Since it is rather laborious and time consuming to determine such properties, these procedures have to be simplified for routine use in the laboratory.

With regard to base composition, the isolation of pure DNA is wearysome, and optimal conditions for cell lysis often have to be found empirically. In addition, a large amount of cells needs to be harvested to gain sufficient DNA.

A simple procedure for the isolation of DNA is described. The resulting DNA is pure enough to allow a reasonably accurate determination of its base composition by the method of Ulitzur (17). Since the DNA base composition of micrococci (65 to 75 mol% guanine plus cytosine [G+C]) differs greatly from that of staphylococci (30 to 40 mol% G+C), even an approximate determination of this value is sufficient to distinguish between these two genera.

To isolate the DNA, the cells of a 300-ml culture grown overnight are harvested and suspended in a small amount (1 ml) of saline-ethylenediaminetetraacetate buffer (0.15 NaCl, 0.1 M ethylenediaminetetraacetate, pH 8.0). Proteinase K (50 µg/ml) is added to eliminate nuclease activity. After being mixed with glass beads (diameter, 0.17 to 0.18 mm) until a viscous consistency is reached, the cells are ground in a cell mill (Vibrogen cell mill, Bühler, Tübingen) for 10 min. The cell constituents are then separated from the glass beads by a wash with 10 ml of saline-ethylenediaminetetraacetate buffer and suction through a coarse, sintered filter.

A 5-ml volume of the filtrate is made 1 M with respect to NaCl by adding 0.3 g of NaCl. A 2-ml volume of a 4% cetyltrimethylammonium bromide (CTAB) solution in 1 M NaCl and 2.0 ml of isopropanol are also added. The mixture is shaken vigorously with 1 volume of chloro-

form-isoamyl alcohol (24:1, vol/vol) until a thick, stable emulsion is formed. After a pre-cooling to 0 °C and centrifugation for 30 min at $12,000 \times g$, the clear upper phase containing the nucleic acids and devoid of interfering pigments is pipetted off, and 2 ml of CTAB solution is added. Upon dilution with 1 volume of distilled water, the CTAB salts of the nucleic acids precipitate and are collected by centrifugation. The pellet is dissolved in a few milliliters of 1 M NaCl, and the CTAB is removed upon being shaken once with 1 volume of chloroform. The sodium salt of the DNA is precipitated out of the aqueous phase by the addition of 0.6 volume of isopropanol and collected by centrifugation. A few milliliters of saline-citrate buffer (0.15 M NaCl plus 0.015 M trisodium citrate, pH 7.0) is layered over the pellet until it becomes translucent. The wash water is then discarded, and the pellet is dissolved in 2 ml of saline-citrate buffer.

The protein content of the DNA preparations is determined by the method of Lowry et al. (12) and is less than 50 µg/ml, i.e., less than 2%. By selectively precipitating the DNA with CTAB (6) and isopropanol (13), the ribonucleic acid and polysaccharide content can also be strongly reduced. In fact, by fractionating our DNA preparation over a Sephadex B4 column by the method of Zdražil et al. (18), only a minimal amount of ribonucleic acid and protein contamination was found. Thus, the DNA is sufficiently pure for an estimation of its base composition from the 245-nm/270-nm absorption ratio by the method of Ulitzur (17).

The DNA base composition of several strains has been examined by the isolation procedure proposed here. The results are listed in Table 1. The deviation in base composition between the proposed method and thermal denaturation values derived from the literature is only ± 3 mol% G+C. Therefore, a distinction between micrococci and staphylococci can easily be made. Moreover, it should be pointed out that the

TABLE 1. Comparison of the base compositions of DNA isolated and determined by the rapid procedure with those determined by classical methods^a

Strain		Rapid procedure (a)		Classical procedure (b)		Difference: a - b
		245/270 nm	Mol% G+C	Mol% G+C	Determined by:	
<i>Staphylococcus aureus</i>	CCM 681	0.8237	30.5	31.5	Boháček et al. (4)	-1.0
<i>Staphylococcus aureus</i>	CCM 2286	0.836	33	30.9-33.5	Boháček et al. (4)	+2.1
<i>Staphylococcus aureus</i> Copenhagen	CCM 2504	0.833	32.5	33.0-33.8	Boháček et al. (4)	-0.5
<i>Staphylococcus haemolyticus</i>	DSM 20263	0.853	36.5	36.4	Schleifer and Kloos (15)	+0.1
<i>Staphylococcus saprophyticus</i>	CCM 883	0.831	32	31.6	Schleifer and Kloos (15)	+0.4
<i>Staphylococcus</i> sp.	CCM 1798	0.835	33	32	Boháček et al. (2)	+1.0
<i>Staphylococcus</i> sp.	CCM 2210	0.821	30	32.0	Silvestri and Hill (16)	-2.0
<i>Staphylococcus capitis</i>	ATCC 27840	0.836	33	35.5	Kloos and Schleifer (9)	-2.5
<i>Staphylococcus simulans</i>	ATCC 27848	0.836	33	34.0	Kloos and Schleifer (9)	-1.0
<i>Staphylococcus warneri</i>	ATCC 27836	0.836	33	34.2	Kloos and Schleifer (9)	-1.2
<i>Staphylococcus hominis</i>	ATCC 27844	0.836	33	33.7	Kloos and Schleifer (9)	-0.7
<i>Staphylococcus epidermidis</i>	ATCC 14990	0.830	32	34.6	Hugh and Ellis (8)	-2.6
<i>Micrococcus luteus</i>	CCM 149	1.03	74.0	74.6	Boháček et al. (1)	-0.6
<i>Micrococcus luteus</i>	CCM 169	1.028	73.5	73.3	Boháček et al. (1)	+0.2
<i>Micrococcus varians</i>	CCM 1395	1.014	70.6	70.6	Boháček et al. (1)	-0.0
<i>Micrococcus varians</i>	CCM 884	1.015	71.5	69	Silvestri and Hill (16)	+2.5
<i>Micrococcus sedentarius</i>	CCM 314	1.0219	72.2	69.3	Boháček et al. (2)	+2.9
<i>Micrococcus lylae</i>	ATCC 27568	1.030	74.0	72.2	Kloos et al. (10)	+1.8
<i>Lactobacillus casei</i> subsp. <i>rhamnosus</i>	ATCC 7469	0.902	45	47	Cantoni et al. (5)	-2.0
<i>Lactobacillus helveticus</i>	DSM 20075	0.873	39	39	Rogosa and Hansen (14)	-0.0
<i>Escherichia coli</i>	ATCC 11775	0.9122	49.2	50	DeLey (7)	-0.8

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CCM, Czechoslovak Collection of Microorganisms, Brno; DSM, Deutsche Sammlung für Mikroorganismen, Göttingen.

deviation of ± 3 mol% G+C is not necessarily a characteristic of the proposed rapid method but may be due to the fact that the literature values are derived from different laboratories. Several examples can be found (11) in which different authors report G+C values that deviate up to ± 5.9 mol% G+C for the same strain despite identical methods of G+C determination.

In our proposed method, the isolation of DNA can be carried out in a test tube after the cells have been broken up with glass beads. Only 5 ml of a concentrated, crude cell extract is required, and the DNA from at least eight strains can be isolated from the cell extracts in about 4 h. Not only is less than 1 g (wet weight) of cells sufficient for DNA isolation, but also no problems with regard to cell lysis arise. In a few carefully chosen steps, DNA can be isolated that is sufficiently free from ribonucleic acid, polysaccharides, and proteins. The determination of the base composition by the method of Ulitzur (17) is accomplished in only a few minutes so that, together with the proposed rapid DNA isolation procedure, an ideal combination for routine determinations of the G+C content of a large number of strains is given.

This method not only is satisfactory for distinguishing between micrococci and staphylococci but also gives equally good results when the DNAs of other organisms, such as lactobacilli or *Escherichia coli*, are compared (Table 1). Thus, this procedure can also be applied successfully to other bacteria.

REPRINT REQUESTS

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