THE SEPARATION OF PLASMID AND CHROMOSOMAL DNA FROM STAPHYLOCOCCUS AUREUS USING POLY-L-LYSINE KIESELGUHR COLUMNS

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Summary. Native DNA from a multiple drug resistant strain of Staphylococcus aureus and sensitive variants derived from it was eluted from poly-L-lysine kieselguhr columns with a linear gradient of NaCl in phosphate buffer. The appearance of satellite peaks in the continuous elution profiles obtained was closely correlated with the presence of plasmid controlled characters in these strains.

The physical autonomy of a Staphylococcus plasmid has been unequivically established by the isolation of an intact plasmid genome (Rush et al 1969). In the present study columns of poly-L-lysine supported on kieselguhr were used to resolve plasmid and chromosomal DNA from strains of Staphylococcus aureus into separate fractions. Fractionation of DNA on poly-L-lysine kieselguhr (PLK) columns has been reported (Ayad and Blamire 1968 ; Helleiner 1969). Ayad and Blamire have concluded that the basis for fractionation on PLK columns is base composition but Helleiner has shown that fractionation is also influenced by molecular weight.

MATERIALS AND METHODS

Bacterial Strains. Staphylococcus aureus strain B (derived from A.T.C.C. strain 14458) used in this study was resistant to penicillin, streptomycin and tetracycline and sensitive to lysostaphin. It produced a deep yellow pigment and the following extracellular enzymes, coagulase DNAase, lipase, staphylokinase, alpha, beta and delta hemolysins. A heat derived variant (strain M) was obtained by growing the parent strain at 42°C (Dunican 1967). Strain M was sensitive to penicillin, streptomycin and tetracycline and resistant to lysostaphin. It had lost the ability to produce yellow pigment and the above mentioned enzymes except alpha and delta hemolysins. An acridine derived varient (Strain 17) was obtained by growing the parent strain in the presence of acridine orange (25 ug/ml). Strain 17 had lost

the ability to produce alpha and delta hemolysins also but was similar to strain M in all other characters so far studied.

DNA preparation. High molecular weight DNA was obtained by the method of Klesius and Schuhardt (1967). The lysostaphin used was a gift from the Mead-Johnson Co.*

Preparation of poly-L-lysine kieselguhr columns. Poly-L-lysine was mixed with kieselguhr and PLK columns were prepared by the method devised by Ayad and Blamire (1968). Columns were fitted with water jackets to give a constant temperature of 15°C. 1,250 ug of DNA was loaded on the columns and these were eluted using a linear molarity gradient of 0.4 molar to 4.0 molar NaCl. The continuous elution profiles were recorded in a Unicam SP500 spectrophotometer using a continuous flow cell and three ml. fractions were collected.

Protein and base assay. Bulked fractions, dialysed against 0.4 M NaCl/KH_2PO_4 buffer and concentrated against polyethylene glycol, were checked for protein content using the method of Lowry (1951). DNA from bulked fractions was hydrolysed and assayed for bases using the method of Wyatt (1951).

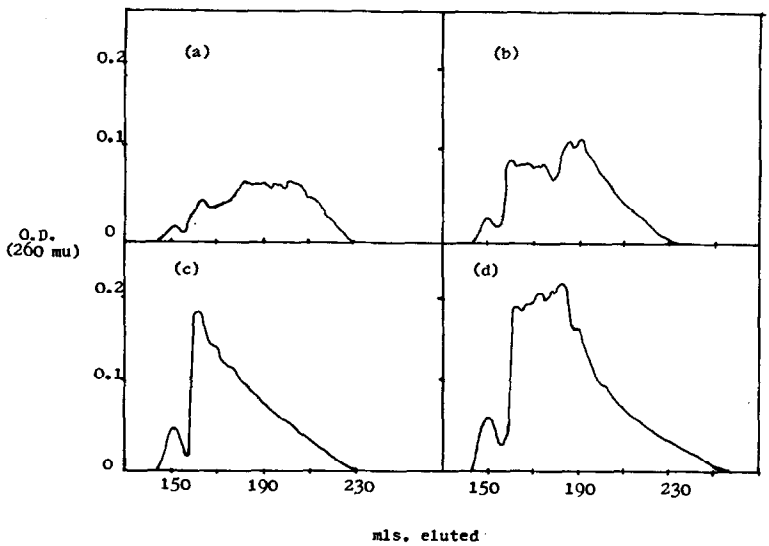

Figure 1. The continuous elution profiles of DNA eluted from PLK columns which were loaded with: (a) 750 ug., (b) 1,000 ug., (c) 1,250 ug., (d) 1,500 ug of native DNA from Staphylococcus aureus strain M (see text)

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RESULTS AND DISCUSSION

The amount of DNA loaded on the column had a marked effect on the elution profiles. Figure 1 shows a comparison of the profiles obtained when 750, 1000, 1,250 and 1,500 ug of DNA from strain M were load on the columns. The loading amount (1,250 ug) routinely used in this work was chosen because at temperatures around 15°C it gave the most uniform profiles and the amount of non-chromosomal DNA present was sufficiently high to give a distinct satellite peak in the profile.

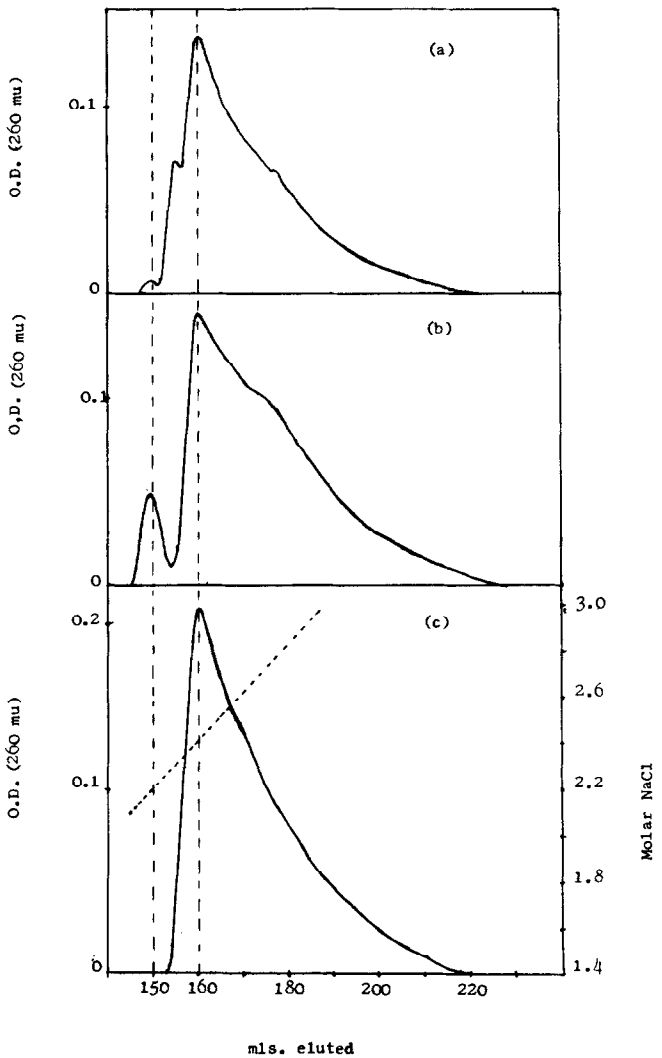

Figure 2. The continuous elution profiles of DNA eluted from PLK columns which were loaded with native DNA from: (a) strain B, (b) strain M, and (c) strain 17 of Staphylococcus aureus (see text)

There was no difference in the quality of separation with new and once used columns. Once a column has been used for fractionation, it can be regenerated by passing 0.4 M buffered saline through it until the ionic strength falls to loading concentration.

Figure 2 shows a comparison of the continuous elution profiles of DNA from the parent B strain and strains M and 17. It can be seen that the parent strain DNA was fractionated into three peaks, eluted at 2.2, 2.35 and 2.42 molar NaCl. The DNA from strain 17 gave a single major peak eluted at 2.42 M NaCl. The DNA from strain M was resolved into two fractions eluted at 2.2 and 2.42 M NaCl.

Figure 3 shows the elution patterns of bulked fractions from the peak of the elution profiles for the original DNA. The fractions corresponding to the main peak and to the satellite peaks, when reeluted from the column after extensive dialysis to remove the NaCl from the DNA were eluted at salt molarities corresponding to the original salt molarities and did not show any

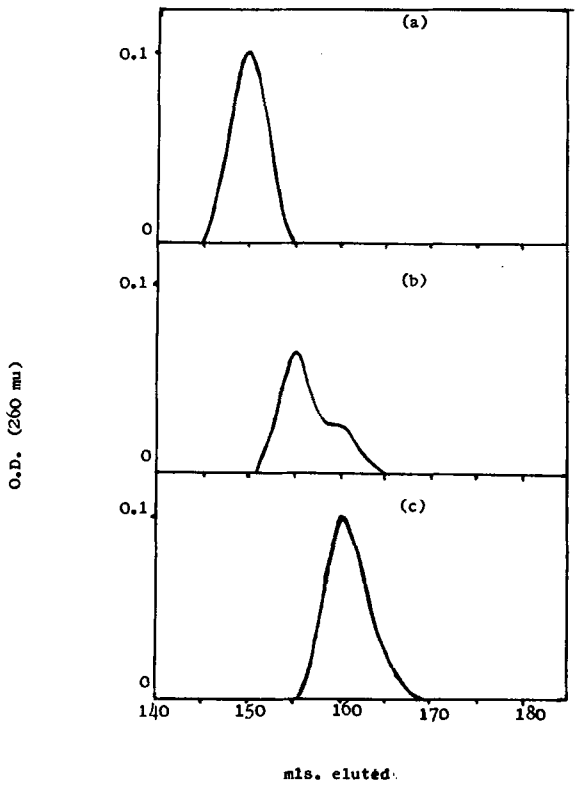

Figure 3. The continuous reelution profiles of DNA from PLK columns which were loaded with DNA previously eluted from PLK columns at (a) 2.2 M NaCl (b) 2.35 M NaCl (c) 2.42 M NaCl.

contamination from the satellite and main DNA components respectively. This indicates that the peak obtained represent distinct components.

A base assay on paper chromatography of the acid hyrolysates of bulked fractions from the main and satellite peaks showed that only adenine, thymine guanine and cytosine were present in all cases. The absence of protein from all peaks was shown by the method of Lowry (1951). These tests indicate that the satellite and main peaks were due only to DNA.

The results presented in Figure 2. confirm our earlier findings (Dunican 1967), that Staphylococcus aureus strain B harbours extrachromasomal characters. The presence of two early satellite peaks suggests that there is more than one plasmid species involved. The presence of at least the penicillinase alpha plasmid and a tetracycline resistance plasmid in a strain of Staphylococcus aureus has been reported. (Richmond et al 1964; Dyke and Richmond 1967). Evidence obtained in our laboratory (unpublished data) from transduction experiments using penicillin and tetracycline markers suggests that there is also at least a penicillin and a tetracycline resistance plasmid present in strain B. A cell harbouring two plasmids has been described as a "plasmid deploid" since in most experiments the genetic markers on the plasmids were similar enough to give rise to a partial diploid cell (Richmond 1969a). It is now possible to distinguish two types of plasmid diploid based on whether the two plasmids belong to the same or a different compatibility group (Richmond 1969b). Plasmids which can co-exist on different sites in the cell give rise to a dissociated diploid situation and plasmids which compete for the same site may form an associated diploid structure. In the light of these considerations it is possible to interpret the two early satellite peaks in the profile of strain B. The first early satellite peak in the profile of this strain is most likely composed of single plasmid DNA. The increased size of an associated diploid structure could give rise to the larger satellite peak on the profile. The single plasmid could be a third independent plasmid or it could be a breakdown product of the double plasmid. Associated diploid plasmids are known to be unstable and to give rise to haploid segregants (Richmond 1967). The presence of a sizeable proportion of haploid segregants in the culture could also explain the first early satellite peak. The single satellite peak in the profile of strain M indicates the presence of a single plasmid. It is significant that this peak is eluted at the same salt molarity as the first small peak in the profile of strain B. This means that the two plasmids have very similar base composition. The plasmid remaining in strain M is thermostable since it has survived the sublethal heat treatment. Strain 17 gave rise to a single major peak only, indicating the absence of plasmid DNA.

This is in accord with the plasmid removing properties which acridine orange is known to have (Watanabe et al 1961). The major peak for all three strains was eluted at the same salt molarity and these being due to chromosomal DNA confirms the close relationship of the three strains.

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