SHORT COMMUNICATION

Gradient Elution from a DEAE-cellulose Column of Capsulate and Non-Capsulate Strains of *Klebsiella aerogenes*

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Klebsiella aerogenes strains A3 and A3(0) which had been grown at 37 °C on nutrient agar medium were applied to a DE-52 cellulose/CC-31 cellulose column and eluted with a linear NaCl gradient in 0.05 M-phosphate buffer, pH 6.0. Characteristic elution profiles were observed: the encapsulated, slime-forming strain A3 gave an elution peak with 200 mM-NaCl and the non-capsulate, non-slimy strain A3(0) with 90 mM-NaCl. These observations may be interpreted in terms of ionic interactions between DEAE-cellulose and components of the cell surface.

INTRODUCTION

It has been found that certain types of bacteria may be separated by elution from a DEAE-cellulose column with a NaCl gradient (Hall *et al.*, 1976). In particular, exponential and stationary phase cells of *Salmonella typhimurium* or fimbriate and non-fimbriate cells of the same organism may be sequentially eluted (Hall *et al.*, 1978). Differences in the affinity of these cells for DEAE-cellulose may be interpreted in terms of the electrical charge carried by the cells.

In view of the presence of glucuronic acid units in the exopolysaccharide of *Klebsiella aerogenes* strain A3 (Wilkinson *et al.*, 1955; Aspinall *et al.*, 1956), it was of interest to compare the chromatographic behaviour of the encapsulated, slime-forming strain A3 (Cap⁺ Sl⁺) with that of strain A3(0) which does not produce either capsules or slime (Cap⁻ Sl⁻; Wilkinson *et al.*, 1954).

METHODS

Materials. Pre-swollen, microgranular DEAE-cellulose (DE-52 cellulose) and unsubstituted cellulose (CC-31) were obtained from Whatman. Starting buffer (SB) was 0.05 M-sodium phosphate, pH 6.0, containing 0.05% (w/v) Tween 80.

Organisms and growth conditions. The strains of *Klebsiella aerogenes* were NCIB 8805 [A3; Cap⁺ Sl⁺] and NCIB 9261 [A3(0); Cap⁻ Sl⁻]. Each strain was grown on nutrient agar (Oxoid, CM3) in four or five Petri dishes at 37 °C for 18 h. Cells were removed from agar surfaces with a small volume of SB, separated by centrifugation (2000 g, 15 min) and washed three times by centrifugation in SB. The cell pellet was finally suspended in 20 ml SB and homogenized for 30 s with a Laboratory Mixer-Emulsifier (Silverson Machines, Chesham, Bucks.) at 600 rev. min⁻¹. The presence of capsules in washed cells of strain A3 and their absence from strain A3(0) were confirmed by microscopic examination of a wet-film in India ink (Duguid, 1951).

Chromatography. CC-31 cellulose powder (14 g) was washed several times with distilled water, by decantation, to remove fines. A slurry of the CC-31 cellulose was then thoroughly mixed with a thick suspension in SB of DE-52 cellulose which had been prepared by precycling DE-52 cellulose (72 g) according to the manufacturer's instructions. The intimate mixture of celluloses was then transferred to a 2.5 cm bore Multisystem column (Whatman) and the column was packed and equilibrated with SB to a constant bed height (approx.

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Fig. 1. Separation of a mixed population of cells of *Klebsiella aerogenes* strains A3 (Cap⁺ SI⁺) and A3(0) (Cap⁻ SI⁻). Cells of strain A3 (7.2 mg) and A3(0) (6.4 mg) were introduced into the column which was developed at a flow rate of 36 ml h⁻¹ with a gradient (○) prepared by mixing limit buffer (600 ml SB containing 100 g NaCl) with SB (1400 ml). The absorbance of fractions at 600 nm was measured (●).

25 cm). A suspension (5 ml) of cells (10 to 25 mg dry wt) in SB was then introduced. Elution was accomplished by means of a linear NaCl gradient in SB; 5 ml fractions were collected.

Analytical methods. The dry weight of cells in the original suspension and in eluted fractions was determined by referring the absorbance at 600 nm to a calibration graph.

RESULTS AND DISCUSSION

Washed cells of *Klebsiella aerogenes* strain A3 were eluted in a single peak with SB containing 200 mM-NaCl; in separate experiments 55% and 60% of the cells applied to the column were eluted. In contrast, cells of strain A3(0) showed an elution peak with 90 mM-NaCl in SB; the efficiency of the elution process was 50% in two experiments.

A population containing similar amounts of cells of strains A3 and A3(0) was prepared by mixing washed cells of the two strains which had been grown separately on nutrient agar. A sample of this mixed population was then applied to a DE-52/CC-31 cellulose column. On development with the NaCl gradient a clear separation of the non-capsulate and capsulate cells occurred (Fig. 1); this was confirmed by microscopic examination of wet-films in India ink of samples taken from the elution peaks. The NaCl concentrations (90 mM and 200 mM) at the elution peaks of the two populations were identical with those determined in the experiments with cells of the individual strains. The recoveries of cells applied to the column were 46.7% for strain A3 and 53.3% for strain A3(0). These recoveries were considered to be satisfactory, especially as in an early experiment with a DE-52 cellulose column only 10% of strain A3(0) cells were eluted; the position of the peak on the gradient in this experiment was again 90 mM-NaCl. Use of CC-31 cellulose in admixture with DE-52 cellulose presumably served to dilute the functional diethylaminoethyl groups, to which the cells bind, thereby reducing the tendency of cells to agglutinate and clump on introduction to the column.

The exopolysaccharide of strain A3 is essentially a linear polymer of D-glucose, D-glucuronic acid and L-fucose units with single glucose groups attached as side-chains to each glucose residue in the main chain; one acetyl group occurs in every eight sugar residues (Aspinall *et al.*, 1956; Conrad *et al.*, 1966). The occurrence of this material in the capsule of strain A3 and its absence from the surface of strain A3(0) (Wilkinson *et al.*, 1954; Salton, 1960) would account for the greater negativity and firmer attachment to DEAE-cellulose of strain A3. These conclusions are also broadly consistent with the observations by James & List (1966) that strain A3(0) cells have a lower electrophoretic mobility at pH 7.0 than those of

A3 but do not support the opinion of these workers that a small amount of capsular material, possibly in the form of a microcapsule, is attached to A3(0) cells.

The viability of the cells was not measured in the experiments described. However, earlier work (Hall *et al.*, 1976) has shown that viable *Escherichia coli* and *Salmonella typhimurium* can be eluted from DEAE-cellulose columns and it is likely that *K. aerogenes* would also be viable.

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