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Note

Behaviour of microbial cells on columns of some types of Sephadex gels

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Because of the widespread use of Sephadex gels in biochemistry, observations on the behaviour of microbial cells in Sephadex columns are of great importance. Often the liquids to be separated by the gel are not sterile, leading to probable microbial growth inside the gel columns. While the Sephadex gel can be protected by certain antimicrobial agents, there is yet little known about the nature of microbial cells in contact with gel particles.

In our experiments, cells of the following strains were used: soil yeast *Rhodotorula glutinis*, the bacteria *Sarcina lutea*, *Escherichia coli*, *Pseudomonas* sp., *Bacillus* sp., and unidentified rods. The cells were washed twice in phosphate buffer, pH 7, and were subsequently suspended in 1 ml of the phosphate buffer and introduced onto the column with the Sephadex gel. The cell concentrations were as follows: $0.72 \cdot 10^6$/ml of *Rhodotorula glutinis*; $0.45 \cdot 10^6$/ml of *Sarcina lutea*; $0.43 \cdot 10^6$/ml of *Escherichia coli*; $9.4 \cdot 10^6$/ml of *Pseudomonas* sp.; $4.06 \cdot 10^6$/ml of *Bacillus* sp.; $0.56 \cdot 10^6$/ml of unidentified rods. G-series Sephadex (G-50, G-100 and G-200), cation exchangers SE C-25 and SM C-25, and anion exchanger DEAE A-25 were used. The cells from the Sephadex G columns were eluted with 0.5% NaCl solution and those from the SE or SM Sephadex columns with phosphate buffer (pH 6); 3.5-ml fractions were collected. Presence of bacterial cells in the fractions was recorded by turbidity measurements (at 540 nm) and microscopic observations. The G-series Sephadex columns were found to retain microbial cells to differing extents depending on the cell types involved. Mobile bacterial cells of the genera *Pseudomonas* and *Bacillus* were liberated, with the result that cells were noted in almost all of the fractions. The cells of soil yeast and *Sarcina lutea* were completely retained in the gel column. Upon bacterial cells being contacted with particles of Sephadex SE C-25, SM C-25 or DEAE A-25, electrostatic charges between the gel particles and the cells assume great importance. As their surfaces are negatively charged, the cells left the column containing the strong acidic cation exchanger Sephadex SE C-25, the particles of which are also negatively charged. Moreover, the elution volume of the cells was the same regardless of cell size and mobility (Fig. 1). Sephadex SM C-25, a weakly acidic cation exchanger, appeared to be selective for the microbial cells tested: although all the cells left the column, the elution volumes of the cells were different (Fig. 2). First to be liberated were the cells of *Bacillus* sp. and *Pseudomonas* sp.; the cells of *Rhodotorula glutinis* were last. This can be explained by a weak adsorption of

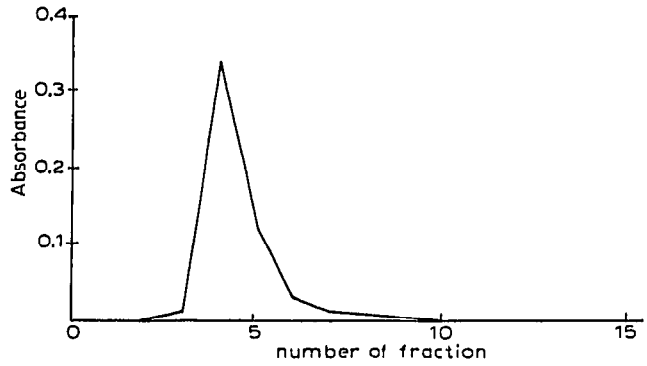

Fig. 1. Elution of microbial cells from a Sephadex SE C-25 column. Eluent, phosphate buffer (pH 6); column dimensions, 30 cm × 1.5 cm; flow-rate, 1.2 ml/min.

the cells on the particles of gel used. Although the experiment shown in Fig. 2 was repeated only twice, the same result had been noted many times previously in experiments performed for other purposes. Upon cells being introduced onto a column with anion gel Sephadex DEAE A-25, complete adsorption of the cells was observed. Eluents such as a 0.1–1.0 *N* NaCl solution and Tris buffer (pH 9) did not liberate the microbial cells from the gel.

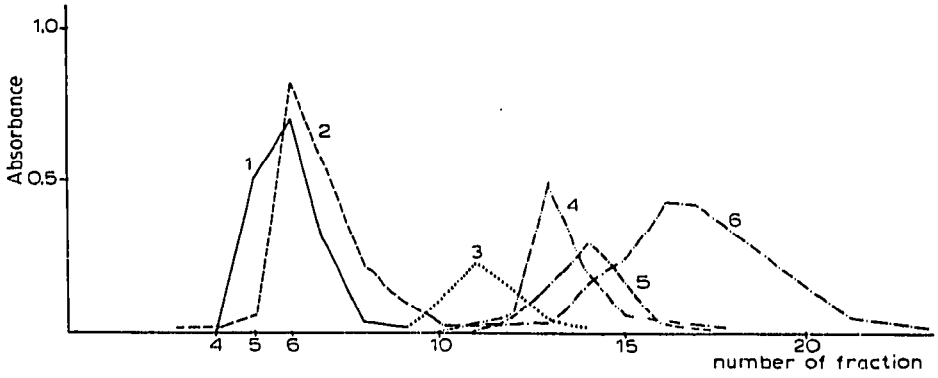

Fig. 2. Elution of microbial cells from a Sephadex SM C-25 column. Eluent, phosphate buffer (pH 6); column dimensions, 22 cm × 1.5 cm; flow-rate, 0.8 ml/min. 1 = *Bacillus* sp.; 2 = *Pseudomonas* sp.; 3 = *Escherichia coli*; 4 = *Sarcina lutea*; 5 = unidentified rods; 6 = *Rhodotorula glutinis*.

This result is different from that obtained by Zviagincev and Guzev[1] and Kurozumi *et al.*[2] in the experiments with anion exchangers of the Dowex type, which appeared to be useful for separating some bacteria from *Bacillus* sp. It is possible that, in the case of Sephadex gel, the liberation of cells depends not only on electrostatic binding but also on gel porosity and size, and the mobility of the microbial cells.

REFERENCES

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2 T. Kurozumi, M. Iton and N. Shibata, *Arch. Biochem. Biophys.*, 109 (1965) 211.