Streptococcus faecalis Chain Disruption

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INTRODUCTION

Many Streptococci are characterized by the formation of long chains of cells. However, *Streptococcus faecalis* forms short chains of 4 to 6 cells or no chains at all, indicating that a cell-separating system must be active throughout the growth cycle. Although biochemically and serologically identical with typical *S. faecalis*, strain NCTC2400 forms long chains. Lominski, Cameron & Wyllie (1958), using Suramin (Bayer 205), stimulated typical *S. faecalis* to grow with long chains which were broken down by filtrates of ordinary cultures.

Toennies *et al.* (1961) noted that the increasing speed of autolysis during exponential growth of *S. faecalis* corresponded to a decrease in average chain length of the cocci. Numerous other workers have also suggested that autolysins may be involved in cell separation (see for example Tomasz, 1968; Chatterjee *et al.* 1969; Fan, 1970*a, b*; Soper & Winter, 1973).

The autolysin in *S. faecalis* occurs in the region of the septum and of the most recently deposited wall, and is produced maximally during the period of exponential growth (Shockman, Pooley & Thompson, 1967; Higgins, Pooley & Shockman, 1970). It is an endo-*N*-acetylmuramidase which hydrolyses the β -1,4 bonds between *N*-acetylmuramic acid and *N*-acetylglucosamine (Shockman, Thompson & Conover, 1967) and may be necessary for the growth and extension of the mucopeptide in the wall (Pooley & Shockman, 1970).

This investigation examines the effect of supernatant fluids from short-chain cultures of *S. faecalis* on the long-chain variant *S. faecalis* NCTC2400.

METHODS

Short-chain *Streptococcus faecalis* strains 064 and Dunnet were isolated in the Department of Bacteriology and Immunology, Western Infirmary, Glasgow from the urine of patients suffering from urinary tract infections. *Streptococcus faecalis* NCTC2400, obtained from the National Collection of Type Cultures, London, grew spontaneously in long chains and was cultured in brain-heart infusion broth (Difco) for 18 h at 37 °C. The culture was centrifuged at 3600 rev./min (2000 *g*) for 20 min and the sediment was washed twice with sterile NaCl (0.15 M, pH 7.0) and finally resuspended in the same diluent to give an EEL nephelometer reading of 80.0 %. Prewarmed brain-heart infusion broth pH 7.4 to 7.6 (500 ml) was inoculated with 10 ml of a 5 h culture of *S. faecalis* Dunnet or 064 and incubated for 18 h at 37 °C. The culture was centrifuged at 3600 rev./min (2000 *g*) and the supernatant fraction (SF) was retained. The SF (1.0 ml) was thoroughly mixed with 0.2 ml of the long-chain suspension of *S. faecalis* NCTC2400 and incubated at 37 °C. At intervals from 0 min to 3 h, the average chain length was determined microscopically and smears were stained to determine any change in the Gram reaction.

For the time lapse photomicrography, 0.02 ml of SF was mixed with a loopful of the long-

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Fig. 1. Time-lapse photomicrographs of chain disruption of *S. faecalis* NCTC2400 by the SF from *S. faecalis* o64: (a) 20 min, (b) 30 min, (c) 40 min, (d) 50 min, (e) 60 min, (f) 70 min, (g) 80 min, (h) the complete sequence of disruption after 90 min. The sites and order of attack are shown by arrows and numbers. The heavy arrows in (a) indicate non-alignment of the chain in the focused plane, and not chain disruption.

chain suspension of *S. faecalis* NCTC2400 on a microscope slide and covered with a coverslip. The slides were incubated in a moist chamber at 37 °C and examined at intervals to determine any alteration in chain length. To assist photomicrography, a drop of 0.2 % Difco agar-solution was added to the mixture to minimize movement of the chains.

RESULTS AND DISCUSSION

The SF disrupted long chains (10 to 40 cells) of *S. faecalis* NCTC2400; 1.0 h after mixing, single cells, paired cells or 4 to 6 cell chains were found. Lysing cells in disrupting chains were also Gram-negative. No disruption of the long chains of cocci occurred if the SF was previously heated at 100 °C for 20 min or if it was mixed with 1.0 % (w/v) Suramin, a known lysozyme inhibitor (Lominski & Gray, 1961).

The chain-disrupting factor in SF did not cause cell separation but disrupted chains by causing a slow lysis of cells randomly distributed within long chains of the *S. faecalis* NCTC2400. There was no apparent change after 20 min exposure to SF, but after 30 min the initial stage of disruption was evident. Further new sites of attack were seen as incubation continued (Fig. 1). There was no such disruption of the long chains by SF which had been heated at 100 °C for 20 min. The chain-disrupting activity of the culture supernate fractions increased during the period of exponential growth, suggesting that the active constituent might be an autolysin.

Fan (1970*a,b*) showed that an autolysin L of *Bacillus subtilis* caused dechaining of long filaments, and suggested that it cleaved the peptide side-chains from the polysaccharide strands of the peptidoglycan thus causing cell separation. However, it is possible that a time sequence examination of this process might reveal lysis of cells within the filament as shown here with *S. faecalis*.

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