APPEARANCE AND ACCUMULATION OF STREPTOCOCCUS FAECALIS CHAIN DISRUPTING/LYTIC FACTOR IN FLUID MEDIA

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SUMMARY: A lytic system is described in S. faecalis zymogenes (Group D) which begins to show in fluid media in exponential phase and attains highest activity before stationary phase. The system disrupts long chains into shorter aggregates by lysing the cocci distributed randomly in the chains

Key Words: Lytic factor, chain disruption, growth curve, exponential phase, optical density.

INTRODUCTION

S. faecalis is known to form short aggregate 2–4 units in fluid media. Abnormally long chain-formation has been reported under the influence of chemically unrelated compounds (1). Long chains, phenotypically formed, or those of atypical strain have been shown to be disrupted by cell-free filtrate of short-chain cultures, indicating the phenomenon to be enzymic, and chain splitting property is shared by egg-white lysozyme (9). Enzyme-defective mutants following UV irradiation have been reported growing into extremely long chains from originally short-chain culture (3). Present study elaborates on emergence and accumulation of lytic/chain disruption on emergence and accumulation of lytic/chain disruption factor in fluid medium and also the location of the system on growth curve.

MATERIAL AND METHODS

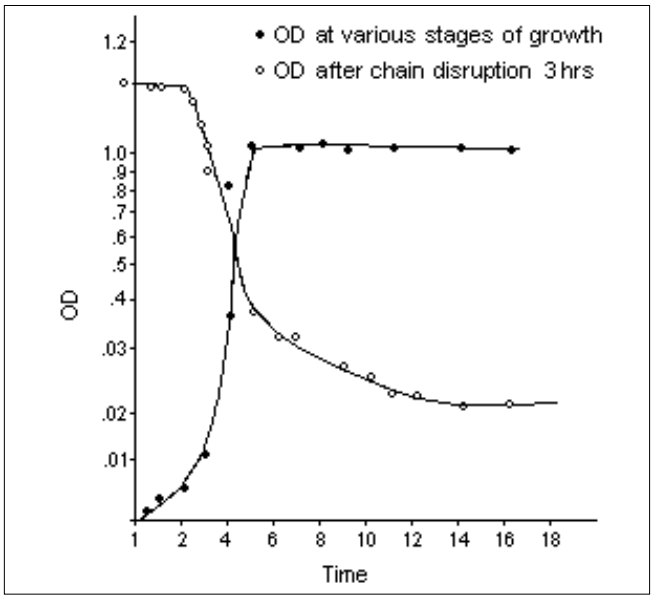
Culture strains and media

Bacterial cultures, media and methodology were used as described by Shaikh (6). 600 ml quantities of medium were inoculated with the short-chain culture 064 and incubated at 37°C. 5 ml aliquots were aseptically removed and optical densities were read on SHIMADZU UV-150-02 Spectrophotometer at wavelength 650 nm. The culture supernates were immediately obtained by centrifuging the samples at 3600 RPM (2000 g) for 15 minutes and decanting them in sterile containers which were stored at 20°C till required. The samples were collected for 18 hours with an interval of 30 minutes. Estimation of chain-disruption/lytic activity was done by the method of Shaikh (6) using uninnoculated BHI broth as control. The OD of each sample was read at the end of the chain-disruption experiment to indicate the lysis of cells at various points on growth cycle.

RESULTS AND DISCUSSION

Short-chain *S. faecalis* 064 followed classical pattern of bacterial growth curve and entered stationary phase in 5 hours time. Optical density thereafter remained more or less the same (Figure 1). Extent of chain disruption/lysis noted is indicated by (Figure 2). The chain disruption activity went parallel with the lytic activities and reached its highest in 12 hours and thereafter remained unchanged. The extent of lysis was concomitant with chain

Figure 1: Gravth sequence of streptococcus faecalis 064 and the action of lytic factor on NCTC 2400.



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disruption. These findings indicated that the lytic factor began appearing 3 hours after inoculation. Lytic/chain disrupting activity gradually increased (Figure 1). Both the activities reached their maximum as culture progressed towards stationary phase. The optical density rose for 5 hours (end of exponential phase) and then remained constant till 18 hours after incubation and likewise was the case with chain distrupting/lytic activity. The maximum lytic/chain disruption activity was noted in samples collected after 12 hours and thereafter. Microscopic examination indicated 70–80% lysis of long-chain cells after 3 hours incubation at 37°C (Figure 2).

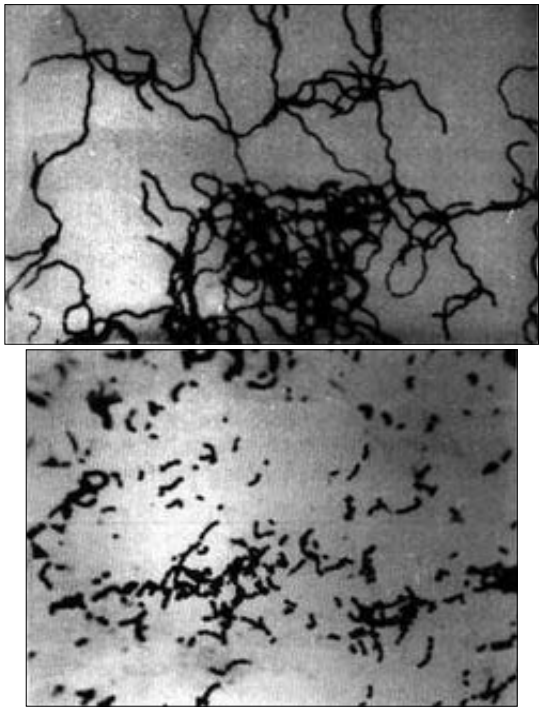

Figure 2: Chain disruption of streptococcus faecalis NCTC 2400. A: Control NCTC 2400, B: Extent of chain disruption after 3 hours. X 1200 gram's staining.

In *S. faecalis* it has been shown that a lytic system becomes noticeable in exponential phase and brings about splitting of long chains. Chain disruption is accomplished by lysing the cells randomly distributed throughout the chain (8). Inhibition and/or inactivation of the system leads to formation of unusually long chains. The short-chain culture supernate readily breaks up the chains into shorter aggregates (3). Partial purification of this system had been reported elucidating an increase of lytic/chain disruption activity with the degree of purification (6). Lytic system has been described in *S. faecalis* ATCC9790 by Shockman *et al.* (9–11). Sanches Pudles *et al.* (7) described the increase in chain length in pneumococcus attributable to absence of enzyme amidase. Indeed, Lominski and Shaikh (2) have described enzyme defective mutants with longer chain length after UV irradiation. Present results conform to earlier findings in relation to lytic factor.

Whether the present enzyme system is identical to that of Schockman *et al.* (4) is difficult to assess, as *S. faecalis* ATCC 9790 has been described as *S. faeciums* by H. M. Pooley, G. D. Shockman, M. L. Higgens and J. Poores Juan.

REFERENCES

1. Lominski I, Cameron J, Wyllie G : Chaining and unchaining of streptococcus faecalis a hypothesis of mechanism of bacterial cells separation. Nature, London, 181:1977, 1958.

2. Lominski I, Gray S : Inhibition of lysozyme by suramin. Nature, London, 192:683, 1961.

3. Lominski I, Shaikh MR : Long chain forming mutants of streptococcus faecalis induced by ultraviolet Irradiation. J Med Microbiol, 1:219, 1968.

4. Pooley HM, Shockman GD, Higgens ML, Porres J : Jaun: Some properties of two autolytic-defective mutants of streptococcus faecalis ATCC 9790. J Bact,109:423, 1972.

5. Shaikh MR : Observations on formation and disruption of chains of bacteria. Ph D Thesis, The University of Glasgow, 1969.

6. Shaikh MR : 1976 Partial purification of streptococcus faecalis chain disruption system by gel filtration. Bl Bkt Hyg I Abt Orig, 236:245-252, 1976.

7. Sanchez-Pudles, Jose, Ronos MC, Garcia JL, Garcia P, Lopez R, Garcia ER : Characterization of a pneumococcal mutant defected in the Ryt A Gene. Eur J Biochem, 158:289-294, 1986.

8. Shaikh MR, Steward-Tull ES : Streptococcus faecalis chain disruption. J Gen Microbiol, 91:195,1975.

9. Shockman CD, Kolb JJ, Toennis G : Relations between bacterial cell wall synthesis. Growth phase and autolysis. J Biol Chem, 230:961, 1958.

10. Shockman CD, Pooley HM, Thompson JS : Autolytic enzyme system of streptococcus faecalis. J Bact, 94:1525, 1967.

11. Shockman CD, Chennry MC : Autolytic enzyme system of streptococcus faecalis. J Bact, 98:1199, 1969.

12. Toennis G, Iszard L, Ragers NB, Shockman CD : Cell multiplication studies with an electronic particle counter. J Bact, 82:857, 1961.

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