Note

Growth Inhibition of a Cytoplasmic Substance of *Streptococcus faecalis* subsp. *zymogenes* against *Bacillus cereus*

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Several strains of lactic acid bacteria are well known to produce antibiotic substances.¹⁻⁴⁾ The authors have recently investigated that effect of cell free extracts from several strains of *Lactobacilli* on growth of *E. coli* and reported that an antibiotic substance was intracellularly produced by *Lactobacillus acidophilus*.^{5,6)} During the course of the investigation, the authors also recognized that cell free preparation of *Streptococcus faecalis* subsp. *zymogenes* showed inhibitory action against *Bacillus cereus*. This communication is concerned with the production of the antibacterial fraction from the cells of *S. faecalis* subsp. *zymogenes*.

For the production of the antibacterial substance, *S. faecalis* subsp. *zymogenes* (ATCC 10100) was cultivated at 30°C for 48 hr in a medium (Medium-A) containing 1% lactose, 0.7% peptone, 0.5% yeast extract, 0.6% Na₂HPO₄ and 0.4% NaH₂PO₄ (pH 6.8). At the end of incubation, the cells were harvested in a centrifuge. The packed cells were suspended, washed in 0.1 M phosphate buffer (pH 7.0) and centrifuged two or more times until a clean supernatant fluid was obtained. The cells were then disintegrated with a sonifer. The sonifer was operated at 40 kHz for 30 min. The unbroken cells and cellular debris were sedimented at $24 \times 10^4 \times g$ for 30 min. The supernatant solution was then filtrated by ultrafiltration fitted with the membrane G-01T (ULVAC). The filtrate obtained was sterilized by passage through Toyo filter pad (85 SA) in the Seitz filter. One milliliter of the aliquot solution was added to 10 ml of a medium (Medium-B) consisting of 1% glucose, 1% bouillon and 0.5% NaCl (pH 6.8) in a test tube. To this medium was inoculated 1 ml of 24 hr' culture of *B. cereus* IFO 3457 grown in the above medium, and incubated at 30°C for 24 hr. Turbidity changes during incubation were determined by measuring of E_{660} . In a control tube 1 ml of sterilized distilled water was used instead of the aliquot solution.

A typical result obtained from the experiment about

FIG. 1. Inhibitory Effect of the Crude Preparation Obtained from *S. faecalis* subsp. *zymogenes* on Growth of *B. cereus*.

B. cereus IFO 3457 was cultivated in Medium-B with (●—●) and without (○—○) addition of the crude preparation.

TABLE I. SPECTRA OF INHIBITORY ACTIVITY OF THE CRUDE PREPARATION OBTAINED FROM *S. faecalis* subsp. *zymogenes*

The procedure for the estimation of growth inhibition against test organisms was the same as that described for *B. cereus* IFO 3457 in the text. Lactic acid bacteria and the other test organisms, respectively, were cultivated in Medium-A and Medium-B at their optimum temperatures for 12 hr.

Test organisms	Growth inhibition
<i>Bacillus cereus</i> IFO 3457	+
<i>Bacillus cereus</i> IFO 3001	+
<i>Bacillus subtilis</i> IFO 3007	— ^a
<i>Bacillus subtilis</i> IFO 3022	— ^a
<i>Escherichia coli</i> IFO 3301	—
<i>Lactobacillus acidophilus</i> IFO 3205	—
<i>Lactobacillus casei</i> IAM 1045	—
<i>Streptococcus lactis</i> IAM 1267	—

+, definite inhibition; —, no inhibition as shown by measuring of absorbance at 660 nm.

^a Results indicating no inhibition of *B. cereus* strains, IFO 3007 and IFO 3022 were considered due, at least in part, to the fact that these strains produced thick slimy materials in the media and turbidity changes of the cultures were difficult to measure.

growth inhibition by this crude preparation against *B. cereus* is shown in Fig. 1, that is, addition of the preparation to the test culture caused an immediate repression against the growth of *B. cereus* (IFO 3457) and turbidity difference between the test culture and the control culture reached maximum at about 8 hr after the beginning of incubation. The antibacterial spectra were shown in Table I. It is readily seen from Table I that antibacterial activity was found against *B. cereus* strains. This crude preparation was very stable at neutral pH and inactive at low pH, and withstood its activity heating at 50°C for 15 min (at pH 7.0) without appreciable loss of activity. By ultrafiltration (G-01T membrane), antibacterial activity was found in the filtrate, but not found in the filtrate when G-05H membrane was used. From this fact the active substance was assumed to have a molecular weight in the range of 500~1000. The active fraction obtained by ultrafiltration was visualized with UV and ninhydrin but not diphenylamine.

In 1954 Hamada isolated a peptide like antibacterial substance from the culture of *S. faecalis*.⁷⁾ However, that substance is essentially different from the preparation obtained in this experiment in their pH sensitivity. In the present experiment antibacterial activity of the cell wall and cytoplasmic membrane prepared from the cells of *S. faecalis* subsp. *zymogenes* was also examined, but no appreciable activity against *B. cereus*

was found in the both preparations.

The experimental details of further purification and chemical properties of the antibacterial substance found in the cells of *S. faecalis* subsp. *zymogenes* will be described elsewhere along with a discussion of the ecological significance of production of the antibacterial substance by *S. faecalis* subsp. *zymogenes* in human gut, because *S. faecalis* strains are known to be one of *Streptococci* which are most frequently found in the gut and faeces of man.⁸⁾

REFERENCES

- 1) A. T. R. Mattick and A. A. Hirsch, *Nature*, **154**, 551 (1944).
- 2) E. B. Collins, *Appl. Microbiol.*, **9**, 200 (1961).
- 3) I. Y. Hamdan and E. M. Mikolajcik, *J. Antibiot.*, **27**, 673 (1974).
- 4) A. L. Branen, H. C. Go and R. P. Genske, *J. Food Sci.*, **40**, 446 (1975).
- 5) A. Hosono and F. Tokita, *Jap. J. Zootech. Sci.*, **48**, 250 (1977).
- 6) A. Hosono, K. Yatsuki and F. Tokita, *Milch-wissenschaft*, **32**, 727 (1977).
- 7) K. Hamada, *Medicine and Biology*, **32**, 195 (1954).
- 8) R. T. J. Clarke and T. Bauchop, "Microbial Ecology of the Gut," Academic Press, 1977, p. 52.