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Isolation of protoplast-bursting factor from pig pancreas

It has been reported that the protoplasts of Gram-positive bacteria are lysed by RNAase (EC 2.7.7.16)[1], lipase (EC 3.1.1.3)[2], trypsin (EC 3.4.4.4)[3], and some bacterial enzyme-like substances[4]. We found that the heat-treated acetone powder of pig pancreas has a strong bursting activity toward protoplasts of *Bacillus megaterium*. In this communication, the isolation of the protoplast-bursting factor is described.

B. megaterium B-151-3 grown at 37° for 5 h in a nutrient medium was collected by centrifugation, suspended in lysozyme solution (0.75 M sucrose, 0.005 M $MgCl_2$, 60 μg/ml lysozyme in 0.01 M phosphate buffer (pH 7.0)), and incubated at 30° for 30 min.

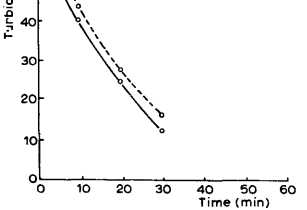

Fig. 1. Effect of enzymes on protoplasts of *B. megaterium*. O—O, pancreas acetone powder, unheated; O---O, pancreas acetone powder, heated; ●—●, unheated trypsin; ●---●, heated trypsin; ×—×, unheated RNAase; ×---×, heated RNAase; –·—·–, control. 0.3 ml of sample (300 μg/ml as protein; determined by KALCKAR's method[6]) was added to 6.0 ml of protoplast suspension, and the mixture was incubated at 37°. As a control, each sample was heated for 15 min in a boiling-water bath.

Protoplast-bursting activity was determined as follows: 0.3 ml of the preparation was added to 6 ml of protoplast suspension, incubated at 37°, and the turbidity of the suspension was determined by the Ito-Beckman type spectrophotometer at 10-min intervals.

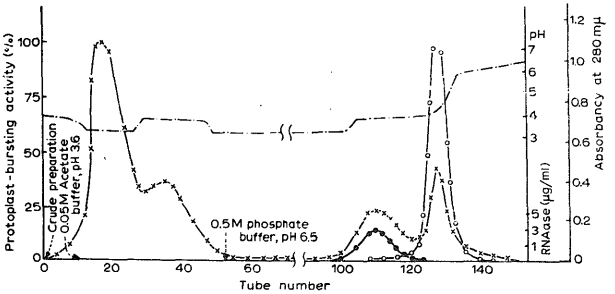

Fig. 2. Chromatographic separation of protoplast-bursting factor with Amberlite CG-50. O—O, protoplast-bursting activity; ×—×, protein (absorbancy at 280 mμ); ●—●, RNAase activity[7], expressed as pure pancreatic RNAase obtained from Nutritional Biochemical Corp.; ·—·—·, pH.

A pig pancreas was homogenized with Waring blendor for 10 min at 16000 rev./min and cold acetone was added. The acetone powder (10 g) was suspended in 100 ml of water, and the insoluble residue was centrifuged off. The supernatant fluid was collected as the crude "Solution".

As shown in Fig. 1, not only the crude "Solution", but also a heat-treated solution (100° for 15 min) decreased the turbidity of the protoplast suspension more rapidly than trypsin or RNAase. This experiment shows that the protoplast-bursting factor in the "Solution" is extremely stable in the pH range between 4 and 5.

The action on the protoplasts was optimal at 37°, and at pH 7.2. The protoplast-

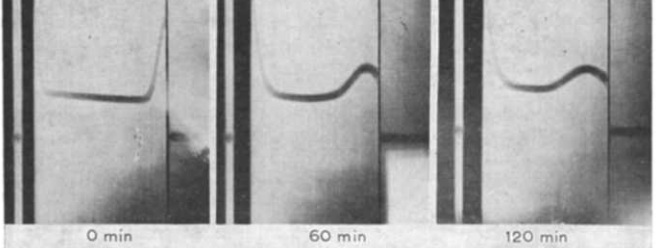

Fig. 3. Ultracentrifugal pattern of purified protoplast-bursting factor. Solvent, phosphate buffer (pH 7.0, I 0.1). Concn., 9 mg/ml; rotor speed, 60000 rev./min; temp., 10°.

bursting factor was not dialyzable and was precipitated with ammonium sulfate (0.5–0.7 satn.).

Purification of the protoplast-bursting factor was carried out as follows: the factor was precipitated with ammonium sulfate (0.5–0.7 satn.) from the crude "Solution", dissolved in 0.05 M acetate buffer (pH 3.5), and chromatographed on an ion-exchange resin (Amberlite CG-50, 200 mesh). The column (2 × 30 cm) was previously equilibrated with 0.05 M acetate buffer (pH 3.5) and eluted with 0.5 M phosphate buffer (pH 6.5).

As shown in Fig. 2, the protoplast-bursting factor was separated from the major fraction of protein. The active fraction was concentrated by ammonium sulfate precipitation, and placed on Sephadex G-75 (medium, 2 × 30 cm), and then eluted with distilled water. After Sephadex treatment, no RNAase activity could be detected in the protoplast-bursting factor.

The ultraviolet-absorption spectrum of this factor indicated a typical absorption pattern of protein with the maximum absorption at 278 mμ.

In the Fig. 3, the ultracentrifugal pattern of the protoplast-bursting factor shows it to be homogeneous.

In the acid hydrolyzate (6 N HCl at 105° for 10 h), the usual amino acids of protein were detected on paper chromatograms.

These results suggest that the factor is a protein. Further discussion will be presented in a later paper.

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