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Note

Rapid isolation of microbial proteases

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Microbial extracellular proteases belong to the most widespread microbial enzymes and there have been many studies of the production, isolation, purification and properties of proteases from various microorganisms.

There are many techniques for the isolation and purification of microbial proteases from culture broth. The methods commonly used are ultrafiltration, solvent and salt precipitation and ion, gel and affinity chromatography. Some mammalian proteases (human urine urokinase[1], trypsin[2] and pancreatic proteases[2]) have been successfully isolated using agar column chromatography. It is shown in this paper that agar can be also used for the isolation and purification of some microbial proteases.

EXPERIMENTAL

Materials

The protease-producing bacterial strain *Bacillus* sp. FB 2 was isolated from soil[3]. Agar No. 3 was obtained from Oxoid (U.K.). Azocasein was prepared in the laboratory[4]. Dry base for the preparation of culture broth was obtained from Imuna (Czechoslovakia). Sodium chloride and other common chemicals were purchased from Lachema (Czechoslovakia).

Chromatography of proteases

A glass column (300 × 12 mm I.D.) was filled with a suspension of agar in water to the height of 220 mm. Agar was then washed with water, 1 *M* sodium chloride solution and water again until the absorbance of the water and salt washings was less than 0.01 at 280 nm in a 1-cm cuvette. After sample application, the ballast proteins were eluted with water. The adsorbed proteases were eluted from the column with 1 *M* sodium chloride solution until no protease activity could be detected in the effluent. The flow-rates ranged from 1 to 2 ml/min. The separation was carried out at laboratory temperature.

Other procedures

The cultivation of *Bacillus* sp. FB 2 was carried out in culture broth for 24 h at 37°C on a rotary shaker. After cultivation, the cells were centrifuged and the clear supernatant was used directly for chromatography.

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The protein content in the eluted fractions was monitored spectrophotometrically at 280 nm, and the Warburg and Christian method[5] was used for quantitative determinations.

The proteolytic activity in the eluted fractions was determined with azocasein as substrate, as described previously[6].

RESULTS

A 10-ml volume of sample (nutrient medium after cultivation) was used for chromatography on the agar column. Fig. 1 shows the distribution of total proteins and the proteases activity in the effluent. Of the applied protease activity, 18.8% was eluted with water together with ballast proteins; 67.3% was eluted with 1 *M* sodium chloride solution in the first 73.5 ml of effluent and 74.8% was eluted in a total volume of 168 ml. The amount of protein associated with proteolytic activity was 2 mg. The specific activity of extracellular proteases increased 81-fold after chromatography.

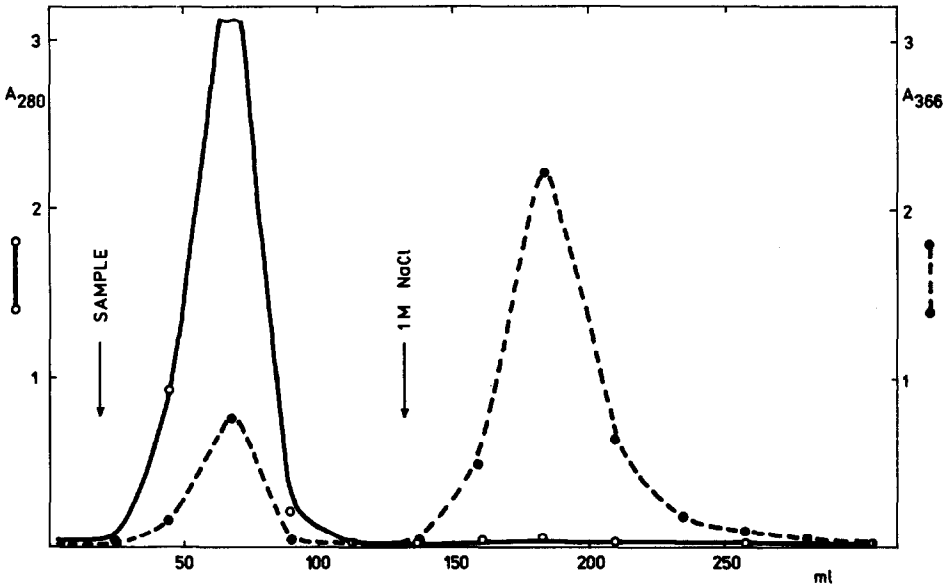

Fig. 1. Chromatography of 10 ml of nutrient medium after cultivation of *Bacillus* sp. FB 2 on a 220 × 12 mm agar bed. The column was washed with water and 1 *M* sodium chloride solution. The flow-rate was 1–2 ml/min. Solid line, absorbance at 280 nm (protein content); broken line, absorbance at 366 nm (proteolytic activity).

DISCUSSION

Agar seems to be a promising sorbent for the one-step separation of some microbial proteases from nutrient medium. Agar is a relatively inexpensive material and can be used for chromatography without any modification. The increase in the specific activity of proteases after chromatography is comparable to that with other affinity chromatographic sorbents.

Agar is not a universal sorbent for the isolation and purification of any microbial protease. In preliminary experiments, milk-clotting protease produced by *Mucor miehei* and extracellular proteases produced by another *Bacillus* sp. strain were not adsorbed on the agar column. This, however, does not reduce the possibility of a wide application of this sorbent.

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