MICROBIOLOGY

Differences in the Mode of Action of Strepzyme and *Helix pomatia* Enzyme Preparations on *Trichotecium roseum* Spores

For the past months, we have been examining the lytic agents affecting yeast and mould cell-walls. Micro-organisms capable of lysing the cell-walls of these organisms were isolated from soil samples. The digestion of the cell-wall material of mould spores, by means of a partially purified enzyme produced by the *Streptomyces GM*, has been described already in a previous communication[1]. Some of us, using the method of digestion by the strepzyme or the gastric juice of the snail *Helix pomatia*, have published several reports on their investigations with protoplasts prepared from various moulds[2–5]. The results obtained clearly demonstrated that protoplasts of *Trichotecium roseum* could be produced by using the lytic enzymes under appropriate experimental conditions.

This communication describes the production of protoplasts from *T. roseum* spores and the differences in the mode of action of the lytic systems used during this investigation. As test organism a young sporulated culture of *T. roseum* was used, growing it at 28° C for 4 days in a medium of the following composition: glucose 10 g ; asparagine 1 g; yeast extract 0·5 g; K_2HPO_4 0·5 g; $MgSO_4.7H_2O$ 0·5 g; $Fe_2(SO_4)_3$ 0·01 g; tap water 100 ml. and distilled water 900 ml.

Spores were recovered from the media by careful shaking with sterile distilled water and sedimentation in a low-speed centrifuge. 0·5 ml. of washed suspensions of spores, adjusted at about 10,000 spores per ml., was incubated with 0·5 ml. of the concentrated lytic enzyme solutions (strepzyme or a gut juice preparation from *Helix pomatia* obtained from L'Industrie Biologique Française) in the presence of 0·1 M phosphate buffer *p*H 6·8 or 0·1 M phosphate–citrate buffer *p*H 5·8, both containing 0·8 M mannitol as a stabilizing agent. The digestion of the cell wall was allowed to take place with gentle shaking at 25° C for 16 h for the liberation of the protoplasts.

Examination under phase-contrast microscope shows that when the gut juice preparation from *Helix pomatia* is used the first visible effect of the enzyme is that the spores become partially detached from the cell wall. The rupture of the cell wall starts at the polar end of the cell that was in contact with the conidiophore and then the other one. The protoplasts can be seen to emerge through the aperture attaining a spherical shape (Fig. 1). The protoplast nature of these structures can be demonstrated simply by dilution of the medium with water when the protoplasts will burst.

On the other hand, when a *T. roseum* spore undergoes the digestive treatment using the strepzyme it was seen that, as previously described, the lytic attack on the cell-wall and the cross-wall is very uniform, giving rise to the formation of two protoplasts which rise together and at the same time from the previous double-dotted cell spore.

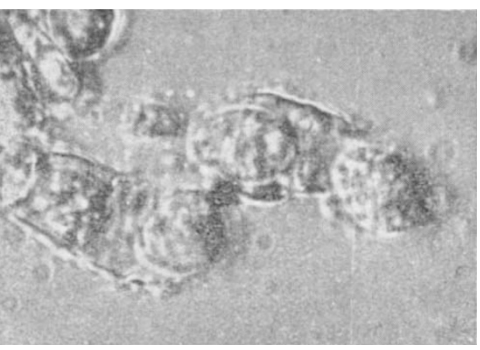

Fig. 1. Shows protoplast formation, after the digestion with the enzyme preparation from *Helix pomatia*. The protoplasts leave the cell-wall at both ends of the double-dotted cell spore of *T. roseum*

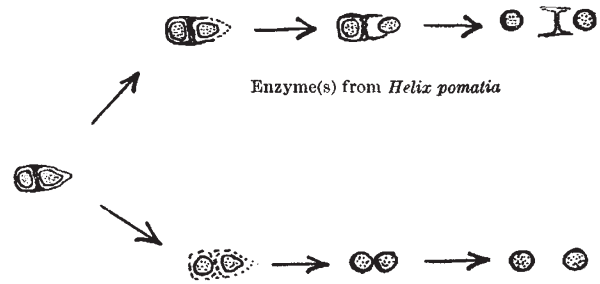

Fig. 2

Fig. 2 shows, diagrammatically, the two different stages in the release of protoplasts from the double-dotted cell spores of *T. roseum*.

These preliminary experiments have shown that the strepzyme produces protoplasts from *T. roseum* spores completely deprived of residual cell-walls which represents a great advantage on the enzyme preparation from *Helix pomatia*.

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A Rapid Method for Determining Phytase Activity of Soil Micro-organisms

During an examination of the breakdown of organo-phosphorus compounds by soil micro-organisms it was found necessary to determine phytase activity in a large number of isolates. The following modification of a recently published technique[1] for the detection of glutamic acid decarboxylase in bacteria proved suitable for this purpose.

Organisms to be examined were grown as streak cultures on glucose–nutrient agar plates incubated at 23°. When sufficient growth had been obtained it was treated with toluene vapour, as described by Stewart[1], and transferred by means of a platinum loop to small tared filter paper disks (7/16 in. diam. Whatman No. 1 filter paper) in 30-mg (wet weight) amounts. A torsion balance was used for rapid weighing in this operation. Organisms (for example, Actinomycetes), the growth of which is difficult to remove from agar, were grown on 'Cellophane' laid on the solidified agar surface. This facilitated removal of the growth and prevented its contamination with agar. After weighing, the disks plus organism were inverted on to glass coverslips and gentle pressure was applied to ensure the formation of a uniform layer of organism between disk and coverslip. Sodium phytate solution (0·01 ml. containing 20 μg phosphorus) was added from an 'AGLA' micrometer syringe (Burroughs Wellcome, Ltd.) to the disks, which were then incubated at 23° for 3 h in a moist atmosphere. Control disks were prepared in exactly the same way except that the sodium phytate was added at the end of the incubation period. In general the *p*H of both sets of disks fell within the range of