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SHORT COMMUNICATION

THE CONTROL OF β-GLUCOSIDASE PRODUCTION IN *TRICHODERMA VIRIDE*

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Abstract—*Trichoderma viride* is capable of adapting to grow on media containing cellulose as the sole carbon source. The production of β-glucosidases and the relative ease of adaption to growth on filter paper is dependent on the form of cellulose used as carbon source.

FOLLOWING observations that the fungal degradation of cotton fibres is brought about by extracellular enzymes (see the review by Hill[1]), it has been demonstrated that more than one enzyme is involved in the process. Reese and his co-workers[2] designated these enzymes C_1 and C_x responsible, respectively, for initial attack on crystalline native cellulose and hydrolysis of modified celluloses. More recently the components of the culture filtrates of *Trichoderma viride* have been separated and shown by Selby and Maitland[3,4] to consist of carboxymethylcellulase, cellobiase and a C_1 component which does not hydrolyse carboxymethylcellulose, cellobiose or cotton. The difficulties of assaying cellulolytic enzymes have been appreciated and discussed by Halliwell.[5,6] It is clear that the use of carboxymethylcellulose as substrate may not indicate cellulase activity. Furthermore it has been suggested that a specific carboxymethylcellulase exists and this is the enzyme which is often assayed. The experiments described below were carried out as part of a programme to prepare a culture filtrate capable of degrading cell walls.

A colony of *T. viride* was obtained from the Commonwealth Mycological Institute, Kew, Surrey, and cultured on potato dextrose agar plates. Liquid cultures were inoculated either by transferring a portion of colony from a plate using a wire loop, or by pipetting a spore suspension. Liquid cultures consisted of 100 ml salts medium[7] supplemented with 1 g of carbohydrate in 250-ml conical flasks which were incubated at 25°. Samples (5 ml) were removed at intervals for assay which was carried out by incubating 1 ml with 5 ml substrate (carboxymethylcellulose, methyl-β-D-glucopyranoside or cellobiose) for 20 min at 25°.

[1] D. W. HILL, *J. Agr. Food Chem.* **13**, 418 (1965).
[2] E. T. REESE, R. G. H. SUI and H. S. LEVINSON, *J. Bacteriol.* **59**, 485 (1950).
[3] K. SELBY and C. C. MAITLAND, *Archs. Biochem. Biophys.* **118**, 254 (1967).
[4] K. SELBY and C. C. MAITLAND, *Biochem. J.* **104**, 716 (1967).
[5] G. HALLIWELL, *J. Gen. Microbiol.* **17**, 153 (1957).
[6] G. HALLIWELL, *J. Gen. Microbiol.* **17**, 166 (1957).
[7] P. R. SAUNDERS, R. G. H. SUIS and R. N. GENEST, *J. Biol. Chem.* **174**, 697 (1948).

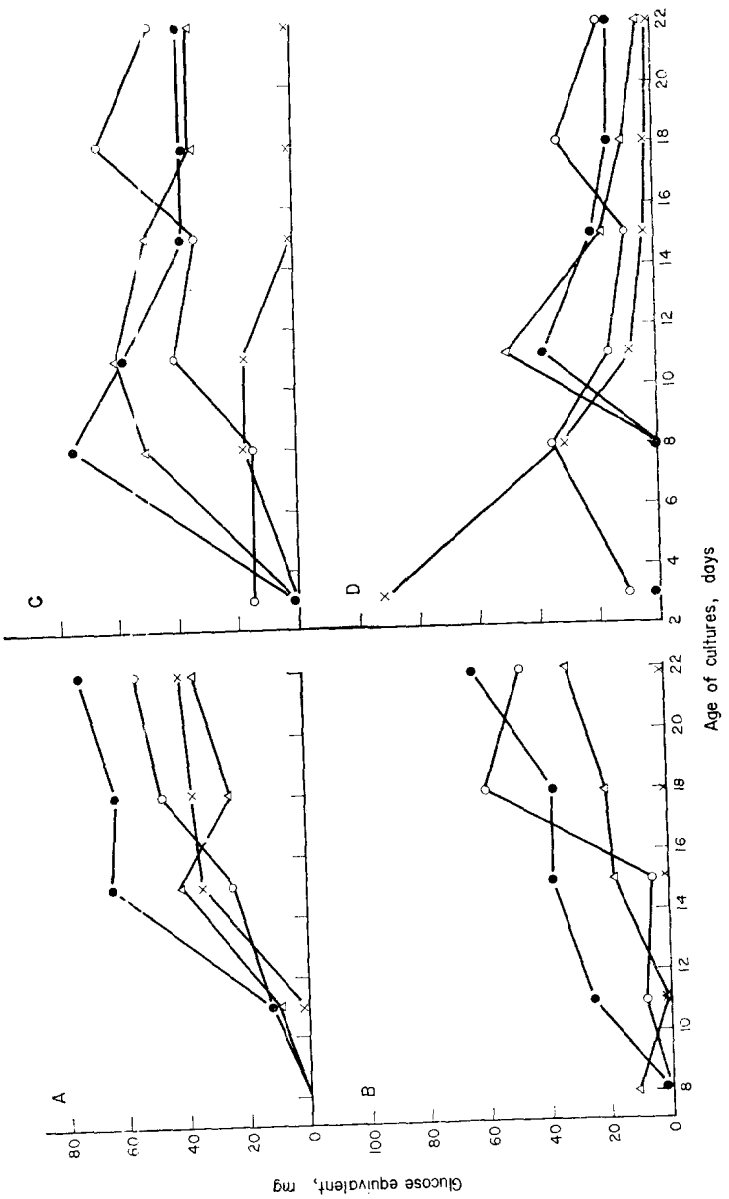

FIG. 1. β-GLUCOSIDASE ACTIVITY OF CULTURE FILTRATES OF *Trichoderma viride* GROWN ON DIFFERENT β-GLUCOSIDES AS CARBON SOURCE.

A, Carboxymethylcellulose; B, Whatman Cellulose powder; C, Methyl-β-D-glucopyranoside; D, Cellobiose.

Reducing sugar was determined by the method of Nelson[8] and glucose using glucose oxidase.[9] Cellulase was assayed by an agar plate method.[10]

The rate of growth of the organism was closely related to the accessibility of carbon source to enzyme attack. Growth was fastest on glucose and no growth was observed with Whatman No. 1 cellulose filter paper as carbon source. Between these extremes the polysaccharide substrates can be arranged in order of decreasing suitability for supporting growth, viz: carboxymethylcellulose, re-precipitated cellulose,[5,6] swollen cellulose[5,6] and Whatman cellulose powder. If mycelia were transferred from these media to fresh medium containing Whatman cellulose filter paper as carbon source the organism established itself and degradation of filter paper began until eventually it disintegrated into small needles or fibres. The ability of the organism to degrade filter paper was related to the form of carbon source of the preceding incubation. Organisms previously grown on glucose grew slowest on filter paper and no signs of degradation of filter paper occurred. Organism cultured on cellulose powder grew and degraded filter paper fastest. Other substrates used as carbon source in order of increasing efficiency were carboxymethylcellulose, re-precipitated cellulose and swollen cellulose. There was thus a close relationship between the state of cellulose in the culture medium and adaptability to growth on filter paper, i.e. more closely the carbon source resembles native cellulose the greater the ease of adaptability of organism to grown on filter paper. Organism grown on glucose as carbon source did not degrade filter paper, nor did culture filtrate show any β-glucosidase activity. In other cases carboxymethylcellulase and methyl-β-D-glucosidase activity follow very similar patterns (Fig. 1), perhaps indicating one enzyme is involved in the hydrolysis of both substrates or that induction of two enzymes is closely related. Cellobiase appeared to behave differently. None of the culture filtrates was capable of degrading cellulose in the agar plate assay. Only in later stages of filter paper degradation did culture filtrates give a positive cellulase result but this was not obtained on every occasion. It is thus evident that *T. viride* adapts to cellulose media. The activities of cellobiase, and of carboxymethylcellulase and methyl-β-D-glucosidase, and possibly also of C_1 and C_x enzymes may be under separate control. The production of enzymes hydrolysing native cellulose is especially interesting because of the solid nature of the substrate and the influence of the preceding conditions of growth on adaptability.

[8] M. NELSON, *J. Biol. Chem.* **153**, 375 (1944).
[9] I. D. FLEMING and H. F. PEGLER, *Analyst* **88**, 967 (1963).
[10] J. G. SAVORY, B. MATHER, C. C. MAITLAND and K. SELBY, *Chem. Ind.* 153 (1967).