

Experimental

4-aceryl-3-(4'-methoxy phenyl) pent-2-ene-1,5-dioic anhydride 3 m.p. 132-133° C, lit.¹ 132° C was prepared as previously described¹ and repeated crystallization from benzene yielded a pure sample for spectral analyses.

4-(4'-methoxy phenyl)-6-methyl oxin-2-one 6, m.p. 113-114° C, lit.¹ 112° C was prepared as previously described and charcoal treatment, followed by crystallization from alcohol, furnished pure sample for spectral analyses.

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**ISOLATION AND CHARACTERISATION OF
 β -SITOSTEROL AND FLAVONOL GLYCOSIDE
FROM THE TRUNK BARK OF *FICUS RUMPHII***

F. rumphii (N.O. Moraceae) is reputed for its medicinal importance¹. In spite of this, practically no work is reported in literature on this plant. The air dried and crushed bark was extracted with 80% methanol. The extract after concentration deposited a yellow solid which on purification gave a white compound A. The supernatant liquid was extracted with ethylacetate. The ethylacetate soluble fraction on purification gave compound B.

Compound A, $C_{29}H_{50}O$, crystallised from ether as white plates was found to be β -sitosterol by TLC, mmp, IR, ms, colour reactions and derivatives formation.

Compound B, $C_{22}H_{22}O_9$, crystallised from ethylacetate and petroleum ether as light yellow solid, m.p. 210° (d). On hydrolysis with 7% ethanolic sulphuric acid it gave sugar and aglycone. The sugar was found to be glucose by TLC and osazone formation.

The aglycone gave colour reactions of flavonoids and showed absorption maxima at 268 nm and 376 nm. It contains only one methoxyl group (Ziesel Method). The aglycone was identified as 3'-methoxy flavonol. Thus the glycoside, $C_{22}H_{22}O_9$, will be 3'-methoxy flavone-3-glucoside.

Our thanks are due to the authorities of U.G.C., New Delhi, for the financial assistance.

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December 10, 1977
Revised Recd. 7-10-1978.

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**A THERMOSTABLE GLUCOAMYLASE FROM
THE THERMOPHILIC FUNGUS *THERMOMYCES
LANUGINOSUS***

HEAT stable enzymes have potential applications in industry. There has been increasing interest in the production of glucoamylase (exo α 1 \rightarrow 4 glucan glucohydrolase) since it directly hydrolyses starch to glucose. This enzyme has been purified and characterised from some species of mesophilic fungi¹⁻³. Subrahmanyam *et al.* have reported on the production of this enzyme from the thermophilic fungus, *Torula thermophila*⁴. We screened some species of thermophilic fungi⁵ for the production of glucoamylase. These were isolated from compost or soil by the method described previously⁶. In this communication we report on the production, purification and some properties of an extracellular glucoamylase produced by the thermophilic fungus, *Thermomyces lanuginosus* ML-M.

The medium used for growing the organisms contained 2% soluble starch (Merck), 0.4% L-asparagine, 0.1% K_2HPO_4 , 0.05% $MgSO_4 \cdot 7H_2O$ and 0.01% (v/v) of a trace element solution⁷. The organisms were grown in 500 ml Erlenmeyer flasks containing 50 ml of the medium in static or in shake cultures at 50° C.

Glucoamylase was assayed by adding 0.1 ml of the culture filtrate to 3 ml of 0.1% soluble starch solution in 100 mM sodium acetate buffer, pH 5.0, and incubating the reaction mixture at 50° C for 30 min. Glucose produced was estimated by the glucose oxidase-peroxidase method⁸.

In static cultures, of the four species of thermophilic fungi tested, *T. lanuginosus* produced the maximum glucoamylase of highest specific activity (Table I). In addition, the enzyme elaborated by this fungus was more heat stable than that of the other fungi. Therefore, *T. lanuginosus* was chosen for detailed studies.

The relationship between the growth of the organism, enzyme production, substrate utilisation and product formation was studied in shake cultures (Fig. 1). Growth was rapid in shake cultures and the maximum growth was obtained when the conversion of starch was complete. The appearance of the

TABLE I
Some characteristics of glucoamylase produced by
thermophilic fungi

Organism	Maximum total activity (units) ^a flask	Specific activity (units/mg protein)	Stability ^b at 60° C
<i>Malbranchea pulchella</i> var. <i>sulfurea</i>	870 (Day 16)	23.9	Retained 70% activity in 6 hours
<i>Talaromyces thermophilus</i>	1125 (Day 11)	33	Retained 60% activity in 6 hours
<i>Thermoascus aurantiacus</i>	340 (Day 14)	21.1	N.D. ^c
<i>Thermomyces lanuginosus</i>	2140 (Day 11)	52.1	No loss of activity in 48 hours

(a) One unit of enzyme is the amount which produces one μ mole of glucose under the conditions of assay as described in the text.

(b) Culture filtrate used as source of enzyme.

(c) Not determined.

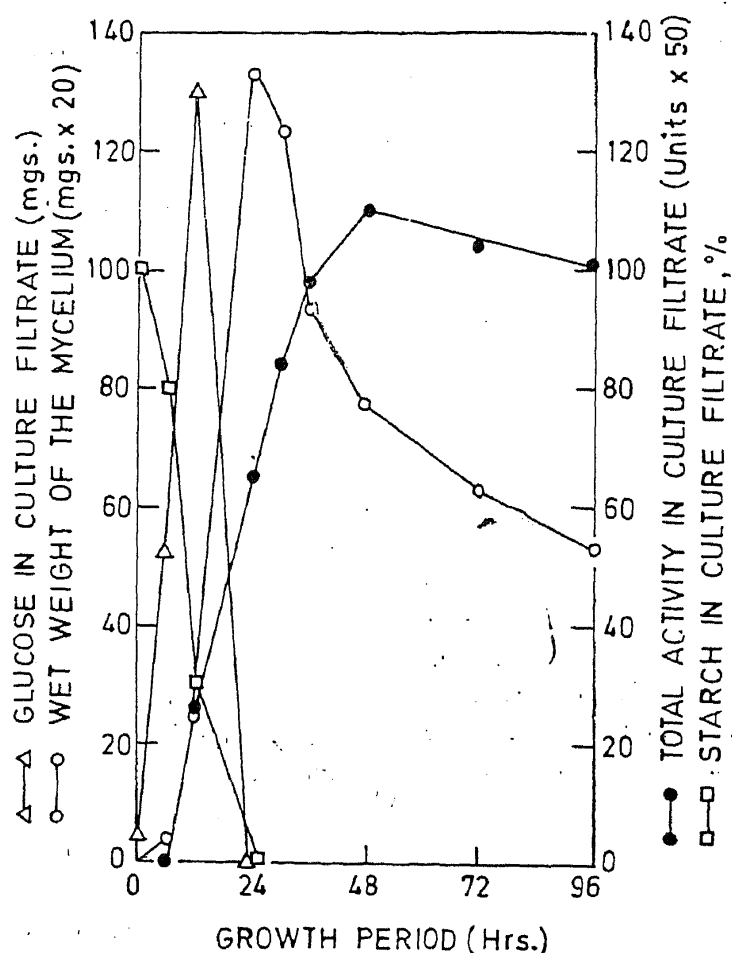


FIG. 1. The relationship between growth, glucoamylase production, substrate utilization and product formation in *Thermomyces lanuginosus* grown in shake cultures.

enzyme in the culture medium increased during the declining phase of the growth. A marked increase in glucoamylase in the culture medium coincided with the disappearance of glucose in the medium. The yield of enzyme was 2.5 fold greater in shake cultures than in static cultures.

The proteins in the culture filtrates of *T. lanuginosus* were precipitated by ammonium sulphate. Glucoamylase in the precipitate was purified by gel-filtration on Biogel P-100, followed by chromatography on diethylaminoethyl cellulose. The final enzyme preparation moved as a single band on polyacrylamide gel electrophoresis at pH 8.3.

The mode of action of purified glucoamylase was studied with maltose, dextrin and soluble starch as substrates. The products of the reaction were identified chromatographically⁹. The enzyme produced only glucose from all the three substrates (Fig. 3) indicating that

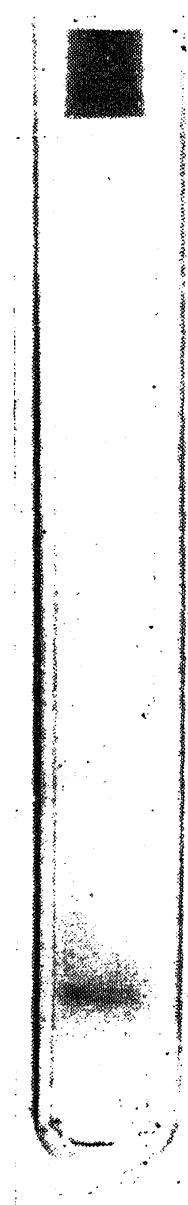


FIG. 2. Polyacrylamide gel electrophoresis of glucoamylase (50 μ g) from *T. lanuginosus* in 7% (w/v) gels (anode towards the bottom of the gel). Coomassie Brilliant Blue R was used for protein staining.

it cleaves successive $\alpha 1 \rightarrow 4$ linkages in the starch molecule. The enzyme cannot hydrolyse dextran, an $\alpha 1 \rightarrow 6$ glucan, indicating that it does not have exo-dextranase activity.

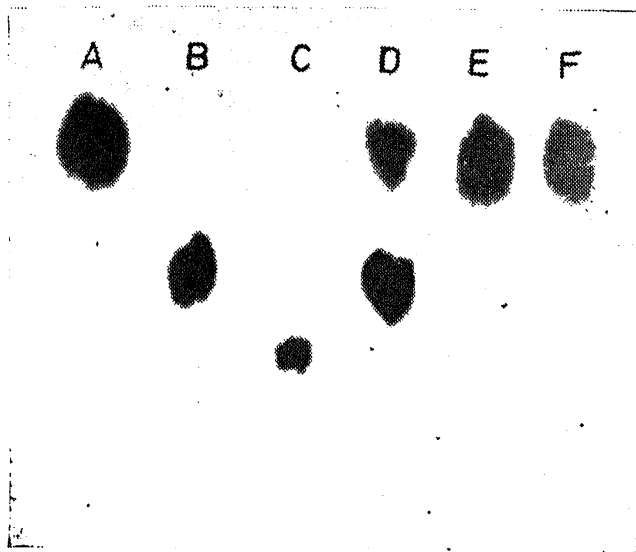


FIG. 3. Identification of reaction products of glucoamylase by paper chromatography. A, B and C are glucose, maltose and maltotriose standards, respectively. D, E and F are the reaction products of glucoamylase using maltose, dextrin and soluble starch as substrates, respectively.

Purified glucoamylase had a pH optimum in the range of 4.0–4.5 and a temperature optimum at 70° C. The enzyme was completely stable for at least one hour at 60° C but it rapidly lost activity above 65° C. Denaturing agents like urea (8 M) or sodium dodecyl sulphate (2.5%) had no effect on the enzyme activity. Guanidine HCl (3 M) inactivated the enzyme by 55%.

The higher yields of glucoamylase from *T. lanuginosus* (Table I) and the thermostability of this enzyme make it suitable for the industrial saccharification of starch. Detailed studies on the physicochemical characteristics of the glucoamylase from *T. lanuginosus* will be published elsewhere.

This investigation was partially supported by grants from the Council of Scientific and Industrial Research to one of us (RM).

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INFECTION OF BANANA LEAF BEETLE BY *BEAUVERIA BASSIANA*

DURING April–May 1977, a large number of banana leaf beetle (*Nodostoma subcostatum*) infesting banana in the horticultural orchard, Assam Agricultural University, Jorhat, were found to be infected by a white muscardine fungus causing death of the pests. White frosty growth of the fungus first emerged through the suture of elytra, thoracic segment and ventral side of abdomen of the dead insect which ultimately covered the whole body (Fig. 1). In most of the cases the beetles were fixed on banana leaves by the fungal mass. The fungus was identified as *Beauveria bassiana* (Bals.) Vuillemin (IMI 215268). The spores are single celled, hyaline, ellipsoidal or oval, 2.5–4.5 × 1.5–2.4 μ . Although the ellipsoidal spores have some affinity with *B. brongniartii* (Sacc.) Petch. clustering of conidiogenous cells is typical of *B. bassiana*.



FIG. 1. A *Beauveria bassiana* infected banana leaf beetle on a piece of banana leaf.