Enzymic synthesis of Oligosaccharides by Penicillium Chrysogenum Q-176.

The enzymic synthesis of oligosaccharides by various organisms including molds, yeasts and bacteria from disaccharides has been reported recently by several workers[1–7]. During the study of the utilisation of different carbohydrates by P. chrysogenum Q-176, it was observed that this strain is capable of synthesising oligosaccharides from the disaccharides

maltose and sucrose. A preliminary survey of the enzymes involved in the synthesis when the mold was grown on different sugars was therefore undertaken and we report briefly the results of these investigations.

The mold P. chrysogenum Q-176 was grown in submerged cultures for six days at 25—26° C. using the medium consisting of minerals as defined by JARVIS and JOHNSON[8]) and different carbohydrates. Both the culture filtrate and the mycelium were tested for enzymic activity. The sugars used for the growth of the mold were glucose, maltose, lactose and sucrose. The mycelium was washed free of broth, dried between folds of filter paper and ground to uniform consistency with water (1:10 w/v on moist basis) and carborundum. The extracts were autolysed under toluene at 0–4° C. for 18 hrs. and centrifuged. The supernatant was used as a source of enzyme. The culture solutions were used as such.

As substrates, representative sugars from the different groups like pentoses, hexoses and disaccharides were used.

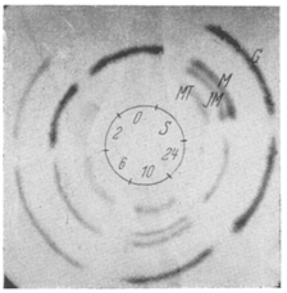

Fig. 1.

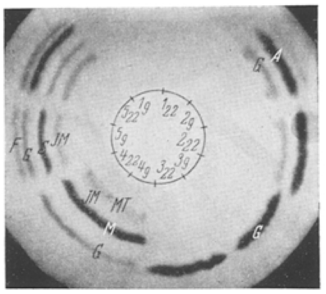

Fig. 2.

Fig. 1. Chromatogram showing the synthesis of isomaltose and oligosaccharides from maltose by the mycelial extract of the maltose grown culture. Numbers indicate time of incubation in hrs. *S* Standard mixture of glucose (*G*); maltose (*M*); isomaltose (*IM*) and maltotriose (*MT*).

Fig. 2. Chromatogram showing the formation of sugars from arabinose (2), glucose (3), maltose (4) and sucrose (5). No. 1 is control without the added substrate. The suffixes indicate the time of incubation in hrs. The mold was grown on sucrose medium. The marked bands are: *A* Arabinose; *F* Fructose; *G* Glucose; *S* Sucrose; *M* Maltose; *IM* Isomaltose; *MT* Maltotriose.

The reaction mixture consisted of 2 c.c. 1% substrate solution, 2 c.c. M/5 acetate buffer (p_H 5·0), and 1 c.c. of enzyme solution. Aliquots of reaction mixture were drawn periodically and the enzyme destroyed by heat. The sugars formed during the reaction were identified by the circular paper chromatographic technique[9]),[10]). Figs. 1 and 2 are typical chromatograms showing the time course of the conversion of the sugars into other carbohydrates. The isomaltose and maltotriose used for identification were prepared in these laboratories by enzymic methods. Isomaltose prepared from the octaacetate which was obtained through the kind courtesy of Dr. A. THOMPSON of the Ohio State University, and maltotriose kindly supplied by Prof. S. PEAT of the University College of North Wales, Bangor, England, were also used for the purposes of identification of the sugars in the hydrolysates.

Among the substrates investigated, synthesis and transformation of sugars were found to occur with maltose, sucrose and arabinose (Fig. 2). The culture solution (with maltose as the carbohydrate source for growth) converts maltose into the disaccharide isomaltose, two trisaccharides (namely maltotriose and one of unknown constitution) and one higher saccharide. The mycelial extract is also capable of synthesising the above oligosaccharides from maltose. Hydrolysis also occurs concurrently with synthesis, resulting in the formation of considerable amounts of glucose. Another observation common to all mycelial extracts is the formation of a band occupying the position of isomaltose on the chromatogram from sucrose, glucose and fructose being obtained as products of hydrolysis (Fig. 2). The partial conversion of arabinose into a sugar occupying the position of glucose by the mycelial extracts has also been observed (Fig. 2). This has been provisionally identified as glucose.

The mechanism of synthesis of these oligosaccharides is presumably one of transglycosidation as proposed by PAN et al[2]).

Further investigations on the isolation and purification of these enzymes and the carbohydrates synthesised by them, are in progress.

Department of Biochemistry, Indian Institute of Science, Bangalore 3 (India),

K. V. GIRI, P. L. NARASIMHA RAO, K. SAROJA and R. VENKATARAMAN.

Eingegangen am 2. Juli 1953.

[1]) PAN, S. C., A. A. ANDREASEN and P. KOLACHOV: Science [Lancaster, Pa.] **112**, 115 (1950).
[2]) PAN, S. C., L. W. NICHOLSON and P. KOLACHOV: Arch. Biochem. a. Biophys., **42**, 406, 421 (1953).
[3]) ROBERTS, H. R., and E. F. MCFARREN: Arch. of Biochem. a. Biophysics. **43**, 233 (1953).
[4]) PAZUR, J. H., and D. FRENCH: J. Amer. Chem. Soc. **73**, 3536 (1951).
[5]) PIGMAN, W. W.: J. Res. Nat. Bur. Standards **33**, 105 (1944).
[6]) BACON, J. S. D., and J. EDELMAN: Arch. of Biochem. **28**, 467 (1950).
[7]) PAZUR, J. H., and D. FRENCH: J. of Biol. Chem. **196**, 265 (1952).
[8]) JARVIS, F. G., and M. J. JOHNSON: J. Amer. Chem. Soc. **69**, 3010 (1947).
[9]) GIRI, K. V., and N. A. N. RAO: Nature [London] **169**, 923 (1952). — J. Indian Inst. Sci. **34**, 95 (1952).
[10]) GIRI, K. V., and V. N. NIGAM: Naturwiss. **40**, 343 (1953).