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BIOLOGICALLY ACTIVE IMPURITIES IN REAGENT GLUCOSE

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We have recently found that reagent grade glucose contains an impurity that is a powerful inducer of the enzyme cellulase for the mold Trichoderma viride QM 6a. This impurity is present in glucose that has been produced by the acid hydrolysis of corn starch (Clinton, Corn Products) but is nearly absent in glucose produced by enzymatic hydrolysis (Staley).

When glucose is passed through a charcoal column and eluted with water, it no longer acts as an inducer of cellulase (Table 1). The inducing material, retained on the column, can be eluted with ethanol. The crude inducer is orcinol-positive and contains several different carbohydrates (paper chromatograms). In amount, it is equal to about 0.15% of the original glucose (Pfanstiehl). The rate of movement of the inducing substance on paper and on carbon columns is in the range of the known glucose disaccharides. This material is an active inducer of cellulase with washed Trichoderma viride mycelium at 5 γ per ml and is at least 200 times as active as cellobiose, the natural inducer (Mandels and Reese, 1960).

There are thirteen possible disaccharides made up of two glucopyranose units. Nearly all are formed by heating glucose in the presence of dilute acid (Thompson et al., 1954; Sowden and Spriggs, 1956; Peat et al., 1958) and most have been reported to be present in hydrol from which glucose has been crystallized (Pigman, 1957). Those that were available have been tested as

Table 1

Cellulase production by Trichoderma viride grown on 0.5% glucose

Source of Glucose	Cellulase units/ml culture	
	as purchased	after charcoal treatment
Pfanstiehl (reagent)	5.7	
J. T. Baker (analyzed)	5.2	0
Merck (USP)	6.7	0
Eastern	8.0	0
Bureau Standards No. 41	0.9	
Corn Products 1st dextrose (Acid)	31.0	0
Clinton 1st dextrose (Acid)	23.0	0
Staley refined dextrose (Enzyme)	0.4	

Methods used in this study are described by Mandels and Reese, 1957, 1960.

inducers. Cellulase is not induced with washed mycelium of T. viride by $\alpha\alpha$ trehalose, $\beta\beta$ trehalose, kojibiose, laminaribiose, maltose, isomaltose, or gentiobiose. It is induced by sophorose (2-O-β-D-glucopyranosyl-D-glucose) at very low concentrations (0.25 γ per ml). It appears probable that sophorose is the active inducer in reagent glucose.

Glucose is perhaps the most widely used substrate in microbiology. The disaccharide impurities are present in it only in very low concentration, but they are difficult to remove by recrystallization. Occasionally, as in this study, the impurities may produce biological effects that are erroneously attributed to glucose itself (Mandels and Reese, 1957). An entirely different impurity present in glucose causes induction of respiratory enzymes in yeast. It has been identified as an oligosaccharide containing 2, 5 anhydro-L-idose (Slonimski et al., 1959). Caution should be exercised in interpreting results obtained with glucose, and indeed with any monosaccharides which have been prepared by acid hydrolysis. Galactose also has been reported to contain disaccharide impurities (Richtmyer, 1958).

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