IN VITRO SYNTHESIS OF OLIGOSACCHARIDES BY THREE "FRUIT ROT" FUNGI*

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ABSTRACT

The influence of component monosaccharide sugars on the *in vitro* synthesis of oligosaccharides by the three fungi, *viz.*, *Macrophoma allahabadensis* Kapoor and Tandon, *Curvularia tuberculata* Jain and *Drechslera australiense* (Bugn.) Subram. and Jain, has been studied. The former two organisms were isolated from the diseased fruits of guava and the last from tomato. The present investigations have shown that the persistence of oligosaccharides could be altered by extra supply of the component sugars in the medium. When maltose was supplied at the rate of 10 g./litre in the medium an oligosaccharide I (R_f 0·18) was synthesized by all the three organisms. An addition of 5 g. glucose to a maltose medium induced the synthesis of an additional oligosaccharide II (R_f 0·30). Thus oligosaccharide (R_f 0·30) was produced only in the culture filtrate of *Macrophoma allahabadensis* and *Curvularia tuberculata*. The addition of D-glucose or D-fructose to a sucrose medium showed no effect on the synthesis of oligosaccharide. The nature of synthesized oligosaccharides was studied partially after subjecting them to total and partial hydrolysis and results have been recorded.

INTRODUCTION

DURING the utilization of some carbohydrates, it was observed by the authors that certain pathogenic fungi were capable of synthesizing oligosaccharides in a sucrose or maltose medium. The exact role of these newly synthesized oligosaccharides and the factors affecting their formation or breakdown has not been properly worked out so far. An attempt has, therefore, been made to examine the influence of component sugars on *in vitro* synthesis of oligosaccharides by three fruit rotting fungi.

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MATERIALS AND METHODS

Mono-conidial cultures of *Macrophoma allahabadensis* Kapoor and Tandon, *Curvularia tuberculata* Jain and *Drechslera australiense* (Bugn.) Subram. and Jain, were employed. The first two organisms were isolated from guava (*Psidium guajava* L.) fruits and the last one from the fruits of tomato (*Lycopersicum esculentum* Mill.). The basal medium consisted of KNO_3, 3·5 g.; KH_2PO_4, 1·75 g.; $MgSO_4$. $7H_2O$. 0·75 g.; and distilled water 1,000 ml. Sucrose and maltose were added singly to this medium so as to provide a carbon concentration of 4 g. per litre. *M. allahabadensis*, *C. tuberculata* and *D. australiense* were grown in media with pH 5·0, 6·5 and 6·0 respectively (Kapoor, 1969). Twenty-five ml. of the medium was poured in each of the 150 ml. Pyrex Erlenmeyer flasks which were sterilized by fractional steam sterilization for 30 minutes on three successive days. The media were inoculated by approximately equal-sized mycelial bits containing spores. The inoculated flasks were incubated at 25° ± 1° C. The other methods were similar to those employed by the authors in their earlier investigations (Kapoor and Tandon, 1969). The newly synthesized oligosaccharides were chromatographically separated from other sugars present in the filtrate with the help of a large sheet of paper (Whatman No. 1). For this purpose the approximate area from the chromatogram (containing the new oligosaccharides) was separated and eluted in 5 ml. of hot water. The solution containing the oligosaccharides was concentrated and a part of it was partially hydrolysed by warming with a drop of dil. HCl for a short period. The other part was completely hydrolysed by heating with a drop of conc. HCl on a steam-bath for one hour. Both the hydrolysed solutions were chromatographically analysed and the component sugars formed after hydrolysis were recorded. R_f values of various sugars were recorded and they have been given at appropriate places in the text. All the experiments were conducted in triplicates.

RESULTS

The results have been summarised in Tables I, II and III.

DISCUSSION AND CONCLUSIONS

Recent discovery of the appearance of synthetic sugars in the medium used by fungi has brought about a drastic change in our concept of hydrolysis of higher sugars. The conventional view of simple hydrolysis has been replaced by a modern concept of "transglycosidation" (or "transglycosylation"). This has been helpful in explaining the formation of syn-

thetic oligosaccharides during the utilization of complex carbohydrates. Fischer *et al.* (1951) have explained the transglycosidation reaction of sucrose. According to Cochrane (1958), "It now appears that at least some and possibly all of the so-called hydrolytic enzymes are in fact group transferases, capable of transferring a sugar residue to any suitable acceptor." In a similar manner higher maltosaccharides could be synthesized from maltose by transglycosidase activity.

TABLE I

Showing the presence of different sugars as well as the persistence of oligosaccharide during the utilization of sucrose; sucrose + D-glucose; and sucrose+D-fructose, by M. allahabadensis, C. tuberculata *and* D. australiense.

Organisms	Sugars	Presence of sugars (days)			
		Sucrose	D-glucose	D-fructose	Oligosaccharide R_f 0·30
M. allahabadensis	Sucrose	0–4	1– 7	1–11	3–5
	Sucrose + D-glucose*	0–4	0–11	1–12	3–5
	Sucrose + D-fructose*	0–5	1– 9	0–15	3–5
C. tuberculata	Sucrose	0–4	4–15	4–15	4–5
	Sucrose + D-glucose*	0–7	0–15	1–15	4–7
	Sucrose + D-fructose*	0–7	3–15	0–15	3–7
D. australiense	Sucrose	0–3	3– 6	3–11	3
	Sucrose + D-glucose*	0–3	0–12	1–13	3
	Sucrose + D-fructose	0–3	2– 7	0–11	2–3

* The quantity added was 5 g. per 1,000 ml. of sucrose medium.

The addition of D-glucose or D-fructose to a sucrose medium had no marked effect on the synthesis of oligosaccharide, but in few cases it

TABLE II

Showing the presence of different sugars as well as the persistence of oligosaccharides during the utilization of maltose and maltose+D-glucose, by M. allahabadensis, C. tuberculata *and* D. australiense.

Organisms	Sugars	Presence of sugars (days)			
		Maltose	D-glucose	Oligosaccharide I R_f 0·18	Oligosaccharide II R_f 0·30
M. allahabadensis	Maltose	0–4	3–7	3–5	..
	Maltose+D-glucose*	0–7	0–12	6–9	6–11
C. tuberculata	Maltose	0–4	3–7	3–5	..
	Maltose+D-glucose*	0–5	0–15	5–7	6–12
D. australiense	Maltose	0–4	4–5	3–4	..
	Maltose+D-glucose*	0–7	0–5	4–7	..

* The quantity added was 5 g. per 1,000 ml. of maltose medium.

persisted for a longer period. The addition of D-glucose to maltose helped the synthesis of an additional oligosaccharide II (R_f 0·30) in the culture filtrate of *M. allahabadensis* and *C. tuberculata*. Bilgrami (1962) also observed an additional oligosaccharide when D-fructose was added to a sucrose medium utilized by *Phyllosticta* spp. The persistence of oligosaccharide I (R_f 0·18) was appreciably enhanced. However, the synthesis of oligosaccharides (I and II) was delayed and the persistence of maltose was prolonged in every case. The synthetic oligosaccharides were consumed by the pathogens earlier than the hydrolytic products, D-glucose and D-fructose. The present results agree with those of Wilson and Lilly (1958), who reported that the new oligosaccharides disappeared from the medium before *Ceratocystis* sp. attained maximum dry weight. They suggested that probably the synthesized oligosaccharides were again hydrolysed and the constituent monosaccharides were ultimately utilised. The present studies establish that, in general, the synthetic oligosaccharides, persist in

TABLE III

Showing the breakdown products of the synthetic oligosaccharides

Carbon source	Oligosaccharide synthesized	Products of partial hydrolysis	Products of total hydrolysis
Sucrose ..	Oligosaccharide (R_f 0·30)	Sucrose D-glucose D-fructose	D-glucose D-fructose
Maltose ..	Oligosaccharide I (R_f 0·18)	Maltose	..
	Oligosaccharide II (R_f 0·30)	D-glucose	D-glucose

the medium for a longer period when component monosaccharides are added to it. Thus the phenomenon of oligosaccharide synthesis is not a reverse process of hydrolysis but merely a deviation from the usual one. After the original sugar had disappeared from the medium the synthetic oligosaccharides were also broken down rapidly. This may explain the persistence of the synthetic oligosaccharides in the medium for a longer period without accumulating in large quantity.

The nature of synthetic oligosaccharides was partially studied (Table III), and it was observed that on complete hydrolysis the synthesized oligosaccharide, formed in sucrose medium, yielded D-glucose and D-fructose. It was further observed that the bands of D-fructose were more concentrated than those of D-glucose. The results were thus similar to those reported by Giri *et al.* (1953), who observed large proportion of D-fructose. It is probable that during the formation of this oligosaccharide there was a transference of fructose residue to the sucrose molecule. Bealing and Bacon (1953) working with enzymatic extracts of *Aspergillus niger* also suggested that formation of synthetic oligosaccharides during the utilization of sucrose was due to the transference of fructose residues to suitable acceptor by a β-fructofuranidase.

The synthesis of oligosaccharide is mainly dependent upon the activity of suitable extra-cellular enzymes. Thus, the quantity and nature of enzymes formed as well as the rate of enzymatic activity would account for the

synthesis of a particular oligosaccharide. The results indicated that their formation depends on the inherent quality and genetic endowments of an organism as well as on the type of sugar involved.

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