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Utilization of Different Carbohydrates by *Cylindrocladium scoparium* Morgan

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The utilization of nine different carbohydrates was studied. An attempt was also made to study the assimilation of mixture of the component units of monosaccharides which are formed during the hydrolysis of oligosaccharides. Glucose, sucrose and maltose were completely utilized within the incubation period whereas fructose, lactose, raffinose, starch and dextrin were detected up to the end of the incubation period. Sucrose and raffinose were utilized through the hydrolytic pathway. The rate of the growth of *C. scoparium* continued to increase up to the end of the incubation period. There was no marked difference in the growth on sucrose and on the mixture of its hydrolytic products; but the mixture of hydrolytic products of lactose supported much better growth than lactose alone. Raffinose was slightly different as it supported better growth at early stage though the final growth was better on the mixture of its hydrolytic products. The sporulation was excellent on glucose, sucrose, maltose, raffinose and dextrin. It varied on others.

Key Words: Carbohydrates, hydrolysis, synthesis, oligosaccharides

Introduction

Carbohydrates play a very important role in the nutrition of fungi. Many investigators have demonstrated clearly that various fungi differ considerably in their mode and rate of utilization of different sugars. Apart from occurring freely as monosaccharides in various fruits, they are also present as component units of different oligo-and polysaccharides. In order to understand the pathway of utilization of mono-, oligo-and polysaccharides, an attempt has been made to undertake chromatographic study on the utilization of some carbohydrates by

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Cylindrocladium scoparium causing fruit rot of tomato. The rate of utilization and the amount of the growth of this organism on oligo-and polysaccharides have also been compared with that obtained on mixtures of corresponding monosaccharides which are known to be formed during their hydrolysis.

Materials and Methods

Cylindrocladium scoparium Morgan causing fruit rot of tomato (*Lycopersicon esculentum* Mill.) was isolated and purified by the usual methods. The basal medium consisted of glucose, 10g; aspargine, 2g; KH_2PO_4, 1g; $MgSO_4.7H_2O$, 0.5g; thiamine, 100 μg and distilled water, 1000ml. The glucose of the basal medium was singly substituted by various mono-, oligo-, and polysaccharides as well as mixtures of different monosaccharides in such a quantity as to furnish 4g of carbon/l. Equal proportion of different components of the mixture was taken in each case. On the basis of previous investigations the initial *p*H of the media was adjusted to 5.5. The media containing monosaccharides were sterilised at 15 lb pressure for 15 min whereas the media containing various oligo- and polysaccharides were sterilised by steaming for 3 successive days. Twenty-five ml of the medium was apportioned in each of 150ml pyrex conical flasks and incubated at 25$\pm$ 2°C for 15 days. The circular paper chromatographic technique of Ranjan et al. (1955) was followed. Presence of starch and dextrin was detected by the iodine test technique. Every day 0.005ml of the culture filtrates from the various sets were placed on the sectored pieces of circular chromatograms at the positions located for the corresponding days. The mycelial growth was harvested after 5, 10 and 15 days on previously weighed Whatman's filter paper No. 42. The filter papers were dried at 60°C for 48 hours, and the dry weight was recorded. An average of triplicate sets was taken as the criterion for growth. The sporulation of the organism was also recorded and it was graded into poor (1–10), fair (11–20), good (21–30) and excellent (above 30) according to number of spores present under low power of microscope. The chromatograms were run in *n*-butanol- acetic acid-water (4:1:5,v/v); while *n*-butanol-pyridine-water(6:4:3,v/v) was used for lactose, raffinose as well as for the mixture of glucose and galactose. These were then sprayed with the reagent prepared from aniline, diphenylamine and orthophosphoric acid (5 vols of 4% aniline in *n*-butanol, 5 vols of 4% diphenylamine in *n*-butanol and 1 vol of orthophosphoric acid). The spots of sugars were developed by heating the chromatograms in an electric oven at 110°C for 90 sec and after comparing the Rf values with those of known sugars the results were recorded in table 1.

Results

It is evident from table 1 that glucose and galactose were utilized in 6 and 7 days respectively whereas fructose was present up to 15th day. Out of the four oligosaccharides used in the present investigation, sucrose was utilised in 11 days. Its hydrolysis into glucose and fructose started on the 2nd day. Both of these hydrolytic products were detected up to 5th day. Maltose was present up to 13th day. Lactose was poorly utilised and it was present up to 15th day. Raffinose was also observed up to 15th day but its partial hydrolysis into melibiose and fructose started on the second day. Melibiose persisted up to 10th day, whereas fructose disappeared after 15th day. Galactose, a hydrolytic product of raffinose, was detected from 10th to 13th day.

There was no marked difference in the growth on sucrose and its hydrolytic

Table 1 *Utilization of different carbohydrates by* Cylindrocladium scoparium *Morgan*

Carbohydrates	Day of incu-bation	Dry wt. (in mg.)	Sporula-tion	Hydrolytic products	Presence of sugars (in days)
	5	39.3	E	—	0–6
D-Glucose	10	55.0	E		
	15	76.3	E		
	5	15.0	P		0–14
D-Fructose	10	42.3	F		(in traces
	15	81.3	F		on 15th day)
	5	61.0	P	—	0–7
D-Galactose	10	73.0	G		
	15	79.0	G		
	5	45.0	E		0–11
Sucrose	10	63.4	E	Glucose	2–5
	15	116.0	E	Fructose	2–5
1/2 Glucose	5	58.6	P	—	0–6
1/2 Fructose	10	69.0	F		
	15	114.0	F		0–15
	5	46.0	E	—	
Maltose	10	69.4	E		0–13
	15	124.1	E		
	5	32.0	G		
Lactose	10	39.2	G	—	0–15
	15	49.6	G		
1/2 Glucose	5	49.3	P		0–4
1/2 Galactose	10	72.0	G	—	
	15	207.0	G		0–15
	5	58.8	E		0–15
Raffinose	10	88.0	E	Melibiose	2–10
	15	88.6	E	Fructose	2–5
				Galactose	10–13
1/3 Glucose	5	38.6	P	—	0–4
1/3 Fructose	10	66.3	F		0–7
1/3 Galactose	15	113.3	F		0–9
	5	44.5	F		
Starch	10	56.1	F	—	0–15
	15	71.7	F		
	5	42.4	E		
Dextrin	10	50.3	E	—	0–15
	15	64.2	E		

P, Poor; *F*, Fair; *G*, Good; *E*, Excellent products (1/2 glucose + 1/2 fructose) but the condition was different with lactose and its hydrolytic products (1/1 glucose + 1/2 galactose) where the rate of growth as well as the final growth was considerably better on mixture of glucose and galactose. The early growth on raffinose (5 and 10 days) was better than on its hydrolytic products (1/3 glucose + 1/3 fructose +1/3 galactose) but the final yield was better on the mixture of glucose, fructose and galactose.

Both the polysaccharides, starch and dextrin, persisted up to 15th day.

The maximum mycelial output was obtained on maltose. The growth of this organism continued to increase up to the end of the incubation period on different carbohydrates.

The sporulation was excellent on glucose, sucrose, maltose, raffinose and dextrin. In other cases it was either good, fair or poor.

Discussion

Cylindrocladium scoparium was capable of utilising all the three monosaccharides included in the present investigation. Glucose and galactose were utilised earlier than the fructose because the traces of the fructose were recorded upto the end of the incubation period. Singh (1972) observed that *Curvularia lunata* utilised glucose, fructose and galactose in 12, 14 and 9 days respectively.

Usually the complex sugars are utilised after being converted into their simple monosaccharide units. Sucrose was partially hydrolysed into glucose and fructose on the 2nd day and the last two persisted up to 5th day. Subsequently they disappeared from the chromatograms. This may be due to their simultaneous utilisation because sucrose was detected up to 11 days. The organisms studied by Tandon and Bilgrami(1957, 1958), Wilson and Lilly (1958), Kapoor and Tandon (1971) as well as Singh (1972) also utilised sucrose after hydrolysing it. There was no difference in the growth of *C. scoparium* on sucrose as well as on its hydrolytic products. Lal and Tandon (1969) working with five isolates of *Colletotrichum gloeosporioides* found that the dry weights of all the isolates were less on a mixture of glucose and fructose than on sucrose alone. No hydrolytic product could be detected during the utilisation of maltose by *C. scoparium*. Similar results were obtained by Ghosh et al. (1965), during the utilisation of maltose by *Colletotrichum papayae* and *Gloeosporium psidii*, but the appearance of synthetic oligosaccharide was an evidence of the breakdown of this sugar. Non-appearance of glucose in those cases was obviously due to its simultaneous utilisation. The same appears to be true in the present case also, even though no oligosaccharide was observed. The organism under study failed to consume lactose within 15 days. In this respect it was similar to *Curvularia lunata* studied by Singh (1972). *C. scoparium* exhibited superior growth on a mixture of glucose and galactose than on lactose. Similar results were obtained by Lal and Tandon (1968) and Srivastava and Tandon (1968) for the fungi studied by them. Raffinose could neither be finished nor hydrolysed completely within 15 days. Melibiose, fructose and galactose were observed as its partial hydrolytic products. According to Cochrane (1958) raffinose produces sucrose and galactose when attacked at α-linkage and melibiose and fructose when attacked at β-linkage. Melibiose was hydrolysed into glucose and galactose. The released quantity of glucose was probably utilised simultaneously and this could not be detected. In this respect *C. scoparium* was similar to *Colletotrichum gloeosporioides* (Ghosh et al. 1965) as well as *Curvularia lunata* (Singh 1972). Final mycelial weight of the organism under study was better on a mixture of glucose, fructose and galactose (1 : 1 : 1) than on raffinose. Kapoor and Tandon (1970) obtained similar results with *Drechslera australiense* and *Curvularia tuberculata*.

C. scoparium showed considerable amount of growth on starch and dextrin but failed to consume these two polysaccharides within the incubation period. Absence of hydrolytic products of starch in the medium was similar to that observed by Kapoor and Tandon (1969) for *Curvularia tuberculata* and *Drechslera australiense*. They had, however, observed glucose as the hydrolytic product during the growth of their organisms on dextrin, but this was not observed in the present case.

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