UTILIZATION AND SYNTHESIS OF OLIGOSACCHARIDES BY SOME PATHOGENIC ISOLATES OF *COLLETOTRICHUM CAPSICI* (SYD.) BUTLER AND BISBY

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Received May 14, 1968

Abstract

Utilization of four oligosaccharides (*viz.*, maltose, sucrose, lactose and raffinose) as well as of the mixture of their hydrolytic products (*viz.*, glucose, $\frac{1}{2}$ glucose + $\frac{1}{2}$ fructose, $\frac{1}{2}$ glucose + $\frac{1}{2}$ galactose and $\frac{1}{3}$ glucose + $\frac{1}{3}$ fructose + $\frac{1}{3}$ galactose) by three isolates of *Colletotrichum capsici* (Syd.) Butler and Bisby obtained from leaf-spots of *Codiaeum variegatum* Blume, *Manihot esculenta* Crantz and *Solanum melongena* L. was studied chromatographically. Sucrose, maltose and raffinose were consumed through a hydrolytic pathway, while none of the hydrolytic product could be detected in the medium containing lactose. The pathogens were also capable of utilizing the hydrolytic products of the oligosaccharides. All of them had synthesized an oligosaccharide during the utilization of sucrose and maltose. They failed to do so during the assimilation of lactose. Only *Manihot* isolate of *C. capsici* synthesized an oligosaccharide, when it was allowed to grow on raffinose. The growth of all the isolates was better on sucrose than on glucose-fructose mixture. They also exhibited better growth on a mixture of glucose and galactose than on lactose. The growth of all of them was inferior on maltose than on glucose. *Codiaeum* and *Solanum* isolates of *C. capsici* grew better on raffinose than on a mixture of glucose, fructose and galactose. *Manihot* isolate of the same species, however, showed more or less identical growth on raffinose as well as on the mixture of its hydrolytic products.

Introduction

Oligosaccharides contain two or more monosaccharides linked together by glycosidic bonds. This glycosidic linkage must be broken before the oligosaccharide may be available for metabolic transformations. Fruton and Simmonds (1958, p. 430) mentioned that this cleavage is effected in biological system with the help of enzymes by two general mechanisms.

The first involves the hydrolysis and the second the phosphorolysis of a glycosidic bond. Most of the pathogenic fungi utilize monosaccharides directly and they are unable to synthesize oligosaccharide when grown on culture media containing simple sugars. On the other hand, when they are grown on complex sugars they not only utilize the oligosaccharides but may also synthesize new oligosaccharides in the media.

In the present investigation, therefore, an attempt has been made to determine (1) the ability of three isolates of *C. capsici* for utilization and synthesis of the oligosaccharides, (2) their pathway of utilization (indirect or direct) as well as (3) the probable effect of hydrolytic products on their growth.

MATERIALS AND METHODS

Single-spore cultures of *Colletotricum capsici* obtained from leaf-spot diseases of *Codiaeum variegatum* Blume (Isolate-A), *Manihot esculenta* Crantz (Isolate-B) and *Solanum melongena* L. (Isolate-C) were employed. They were grown on a number of media and on the basis of the results it was decided to use glucose, 10·0 g.; KNO_3, 3·5 g.; KH_2PO_4, 1·75 g.; $MgSO_4.7H_2O$, 0·75 g. and distilled water, 1000 ml. as the basal medium. In order to study the effect of different oligosaccharides (*viz.*, maltose, sucrose, lactose and raffinose) and the mixture of their hydrolytic products (*viz.*, glucose; $\frac{1}{2}$ glucose + $\frac{1}{2}$ fructose; $\frac{1}{2}$ glucose + $\frac{1}{2}$ galactose and $\frac{1}{3}$ glucose + $\frac{1}{3}$ fructose + $\frac{1}{3}$ galactose) they were substituted singly in place of glucose and their quantity was so adjusted as to furnish 4 g. of carbon per litre. The most suitable pH for isolates A, B and C was found to be 6·0, 5·0 and 5·5 respectively. The pH of media for subsequent studies was adjusted to the most suitable level in each case. The pathogens were grown in 150 ml. conical flasks and were incubated at 25 ± 1° C. 25 ml. of medium was poured in each flask. The medium containing oligosaccharides was fractionally sterilized by steaming for half an hour for three successive days, while the medium containing monosaccharides was autoclaved at 15 lb. pressure for 15 minutes. The experiments were conducted in triplicate sets. Every day 0·005 ml. of the medium from a flask belonging to a particular set was analysed by the circular paper chromatographic technique mentioned by Ranjan *et al.* (1955). The chromatograms of lactose and raffinose were run in *n*-butanol-pyridine-water in the ratio of 6:4:3 (V/V), whereas the solvent used for others was *n*-butanol-acetic acid-water in the ratio of 4:1:5 (V/V). After drying the chromatograms were sprayed with aniline-diphenylamine phosphate reagent (5 vols. 4% aniline, 5 vols. 4% diphenylamine and

1 vol. orthophosphoric acid; Buchan and Savage, 1952). The bands were developed by heating the chromatograms at 110° C. for 90 seconds.

In each case the fungal mats were harvested, after 5, 10 and 15 days of incubation, on previously dried and weighed Whatman's filter-papers No. 42. Simultaneously the pH of the filtrate was also determined. The filter-papers were then dried to a constant weight at 60 ± 2° C. and were reweighed after cooling in a desiccator. The average dry weight of mycelium was taken as the criterion for growth.

Observations

The details of the results have been recorded in Tables I–IV.

Discussion and Conclusions

1. *Assimilation of sucrose.*—The spores of the fungus *Myrothecium verrucaria* (Mandels, 1954) did not use sucrose through a hydrolytic pathway. In the present study all the isolates completely utilized this sugar through hydrolytic pathway within 5–6 days. This indicated that the organisms were capable of producing sucrase or transfructosidase enzyme in sufficient quantity. The hydrolytic products (*viz.*, glucose and fructose) appeared in the medium in each case but glucose was used up earlier than fructose. Similar results were obtained when they were grown on a mixture of glucose and fructose. All the isolates synthesized an oligosaccharide during the utilization of sucrose. A simultaneous synthesis of oligosaccharide along with the hydrolytic products, in the medium containing this sugar, has also been reported by Bealing and Bacon (1953), Giri *et al.* (1954), Tandon and Bilgrami (1957, 1958), Wilson and Lilly (1958), Chandra (1961), Chaturvedi (1961), Ghosh (1964), Ghosh *et al.* (1965) and Lal (1967). The behaviour of *Aspergillus* spp. (Mehrotra and Agnihotri, 1961) and *Colletotrichum inamdarii* (Hasija, 1965) was somewhat different as they failed to synthesize any oligosaccharide on this medium.

The formation of synthetic oligosaccharides during utilization of sucrose is considered to be interesting. Modern concept of transglycosidation has rendered its help in explaining such process. It has been assumed that by the action of an enzyme the glycosidic link breaks up and one of the component units combines with enzymes to form a complex. From this complex the sugar residue is transferred to water or another molecule of the original oligosaccharide. In the later case a higher oligosaccharide is formed.

TABLE I

Showing average dry weight (mg.), final pH and presence of different sugars during the utilization of sucrose and a mixture of glucose and fructose by different isolates of C. capsici

Organism	Sugars	5 days		10 days		15 days		Presence of sugars (days)			
		Dry weight	Final pH	Dry weight	Final pH	Dry weight	Final pH	Sucrose (Rf. 0·41)	Glucose (Rf. 0·59)	Fructose (Rf. 0·63)	Oligosaccharide-I (Rf. 0·35)
Isolate A	Sucrose	44·0	5·5	101·0	6·7	88·0	7·3	0–5	2–7	3–10	2–6
	½ glucose + ½ fructose	19·0	5·8	39·6	5·8	59·3	6·1	..	0–11	0–15	..
Isolate B	Sucrose	40·0	6·1	119·3	6·4	105·8	7·0	0–5	2–7	2–10	2–6
	½ glucose + ½ fructose	19·0	5·5	71·0	5·8	51·5	7·0	..	0–8	0–14	..
Isolate C	Sucrose	37·0	5·5	96·5	6·7	93·0	7·3	0–6	3–9	3–10	3–6
	½ glucose + ½ fructose	18·0	5·5	58·0	5·8	68·3	7·0	..	0–10	0–14	..

TABLE II

Showing average dry weight (mg.), final pH and presence of different sugars during the utilization of lactose and a mixture of glucose and galactose by different isolates of C. capsici

Organism	Sugar	5 days		10 days		15 days		Presence of sugars (days)		
		Dry weight	Final pH	Dry weight	Final pH	Dry weight	Final pH	Lactose (Rf. 0·43)	Glucose (Rf. 0·61)	Galactose (Rf. 0·57)
Isolate A	Lactose	9·0	5·8	16·0	6·7	37·6	7·0	0–15	..	..
	½ glucose + ½ galactose	16·0	5·2	41·6	5·5	45·0	7·0	..	0–10	0–15
Isolate B	Lactose	8·0	5·5	26·3	6·7	43·6	7·3	0–15	..	..
	½ glucose + ½ galactose	21·6	5·5	51·0	6·4	63·6	7·0	..	0– 9	0–15
Isolate C	Lactose	6·0	5·8	22·2	6·1	26·0	6·1	0–15	..	..
	½ glucose + ½ galactose	27·5	5·8	46·6	7·0	58·0	7·3	..	0– 6	0–13

TABLE III

Showing average dry weight (mg.), final pH and presence of different sugars during the utilization of maltose and glucose by different isolates of C. capsici

Sugar	5 days		10 days		15 days		Presence of sugars (days)		
	Dry weight	Final pH	Dry weight	Final pH	Dry weight	Final pH	Maltose (Rf. 0·40)	Glucose (Rf. 0·59)	Oligosaccharide-I (Rf. 0·28)
Maltose	22·1	5·8	75·0	6·4	70·0	7·3	0–13	..	4–13
Glucose	59·1	6·7	118·5	7·0	109·4	7·3	..	0– 7	..
Maltose	42·7	5·8	77·0	6·4	70·5	7·3	0–12	..	4–12
Glucose	43·3	6·7	105·0	7·0	98·0	7·3	..	0–10	..
Maltose	36·0	5·8	61·0	6·4	68·0	7·3	0–10	8–11	4–10
Glucose	49·3	6·7	102·4	7·0	94·8	7·3	..	0– 8	..

TABLE IV

Showing average dry weight (mg.), final pH and presence of different sugars during the utilization of raffinose and a mixture of glucose, fructose and galactose by different isolates of C. capsici

Sugar	5 days		10 days		15 days		Presence of sugars (days)					
	Dry weight	Final pH	Dry weight	Final pH	Dry weight	Final pH	Raffinose (Rf. 0·31)	Melibiose (Rf. 0·35)	Fructose (Rf. 0·65)	Galactose (Rf. 0·57)	Glucose (Rf. 0·61)	Oligosaccharide-I (Rf. 0·23)
Raffinose	20·1	5·8	54·2	6·1	62·0	7·3	0–8	..	6–10	6–12	6–7	..
⅓ glucose + ⅓ fructose + ⅓ galactose	10·0	5·8	42·0	6·1	47·3	6·4	..	..	0–15	0–15	0–9	..
Raffinose	25·3	5·8	69·6	6·1	63·9	7·3	0–5	..	4–10	4–10	4–6	4–5
⅓ glucose + ⅓ fructose + ⅓ galactose	15·0	6·1	54·6	6·7	65·0	7·0	..	..	0–15	0–15	0–8	..
Raffinose	18·2	5·8	58·3	5·8	64·8	7·3	0–9	..	4–10	4–12	5–7	..
⅓ glucose + ⅓ fructose + ⅓ galactose	12·0	5·8	30·6	6·7	48·0	7·0	..	..	0–15	0–15	0–8	..

The dry weights of all the pathogens on sucrose were maximum on the 11th day, after which they decreased. Isolate-B also behaved in a similar manner on glucose-fructose mixture while continuous increase in dry weights was observed in the remaining isolates upto the end of the incubation period.

Working with three isolates of *Colletotrichum gloeosporioides* and two isolates of *C. dematium*, Ghosh (*l.c.*) noticed close similarity in dry weight results on sucrose medium and on a mixture of glucose and fructose. In the present study all the isolates exhibited inferior dry weights on glucose-fructose mixture than on sucrose. Lal (*l.c.*) also obtained similar results with five isolates of *C. gloeosporioides*. The results are in agreement with the remarks of Lilly and Barnett (1951, p. 133), " A complex carbohydrate and its hydrolytic products are not necessarily equivalent in all respects".

2. *Assimilation of lactose.*—The enzyme lactase, β-galactosidase has been reported to cause splitting of lactose into glucose and galactose. Wallenfels (1951) noted the presence of the above enzyme in *Aspergillus oryzae*. He also reported that the above organism synthesized an oligosaccharide on lactose medium.

There was continuous increase in the dry weight upto the end of the incubation period. Chromatographic analysis of the medium also revealed that in every case lactose was present in the medium upto the end of the incubation period of 15 days, but the decrease in the intensity of the band indicated that it was utilized slowly. Chandra (*l.c.*), Chaturvedi (*l.c.*) and Ghosh *et al.* (*l.c.*) had also observed the presence of lactose upto 15 days. *C. gloeosporioides* (Prasad, 1963) and *C. inamdarii* (Hasija, *l.c.*) utilized it in 14 and 12 days respectively.

Lal (*l.c.*), while working with *Manihot* isolate of *C. gloeosporioides*, found that galactose—the hydrolytic product of lactose—was present in the medium and this indicated that it has been utilized through a hydrolytic pathway. In the present study all the isolates failed to produce the hydrolytic products in sufficient quantity. This may be due to slow breakdown of lactose and simultaneous utilization of its hydrolytic products.

The present isolates could not synthesize any new oligosaccharide on media containing lactose. These were synthesized by *Curvularia lycopersici* (Kakkar, 1964) as well as *Curvularia verruculosa, C. pallescence* and *C. fallax* (Srivastava, 1965).

When the organisms were grown on a mixture of glucose and galactose, the former was consumed earlier than the latter in every case. As on lactose

alone, the dry weights of the mycelial mats showed an increase upto the end of the incubation period. They, however, exhibited superior growth on a mixture of glucose and galactose than on lactose. Three isolates of *C. gloeosporioides* and two isolates of *C. dematium* (Ghosh, *l.c.*) developed similar dry weights on lactose and on a mixture of glucose and galactose. Lal (*l.c.*) observed superior growth of *Mussaenda* isolate of *C. gloeosporioides* on lactose than on glucose-galactose mixture. Thus the behaviour of different species as well as isolates of the same species varied considerably in this respect.

3. *Assimilation of maltose.*—Maltose was utilized by all the isolates through hydrolytic pathway. Although glucose, the hydrolytic product could be detected only in the case of isolate-C, but an oligosaccharide was synthesized in every case. This indicated the breakdown of this sugar. Non-appearance of glucose in those cases may be due to its simultaneous utilization or to its conversion to an oligosaccharide. The breakdown of maltose indicated the presence of α-glycosidase in all the isolates of *C. capsici.*

Certain other investigators including Tandon and Bilgrami (1957, 1958), Wilson and Lilly (*l.c.*), Wilson (1960), Mehrotra and Kumar (1962), Agnihotri (1962), Prasad (*l.c.*), Bilgrami (1964) and Ghosh *et al.* (*l.c.*) have also reported the synthesis of one or two oligosaccharides during the utilization of maltose by different fungi. *C. capsici* (Chaturvedi, *l.c.*) and *C. inamdarii* (Hasija, *l.c.*), however, failed to synthesize any oligosaccharide on maltose medium.

The dry weight of isolate-C showed slight increase after 10 days of incubation on maltose, whereas at the end of the incubation period the remaining isolates exhibited a decrease in dry weight on maltose as well as on glucose. All of them showed better growth on glucose than on maltose. Tandon and Chandra (1962) obtained similar results with *Colletotrichum gloeosporioides* and *Curvularia penneseti.* Mehrotra and Agnihotri (*l.c.*) working with five different species of *Aspergillus* recorded better growth on maltose than on glucose.

4. *Assimilation of raffinose.*—A molecule of raffinose is composed of three monosaccharides: glucose, fructose and galactose. When it is acted upon by an α-glycosidase, raffinose breaks up into sucrose and galactose, while β-glycosidase splits it into melibiose and fructose.

Chromatographic analysis of the culture medium indicated that all the three isolates finished raffinose in different periods. Isolates A, B and C consumed it in 8, 5 and 9 days respectively. During their growth on raffinose

all the three isolates produced glucose, galactose and fructose. Melibiose or sucrose were not detected in the medium. It was, therefore, not possible to establish the actual pathway of its utilization. Similar results were obtained by Ghosh *et al.* (*l.c.*) for *C. papayae*.

The results obtained by other investigators show much diversity in the utilization of raffinose by different organisms. Formation of melibiose and fructose has been observed during the utilization of raffinose by *Ceratocystis fimbriata* and *Thielaviopsis vasicola* (Wilson and Lilly, *l.c.*), *Pestalotia banksiana* and *P. citri* (Tandon and Bilgrami, 1958), *Cercosporina ricinella*, *Colletotrichum gloeosporioides* and *Curvularia penneseti* (Tandon and Chandra, *l.c.*), *Botryodiplodia ananassae* and *Macrophomina phaseoli* (Bhargava, 1962) as well as *Phyllosticta* spp. (Bilgrami, 1963). Ghosh (*l.c.*) working with different isolates of *Colletotrichum gloeosporioides* and *C. dematium* reported that only one hydrolytic product, galactose, was detected in all cases. *C. inamdarii* (Hasija, *l.c.*) could produce sucrose and galactose in the medium. Working with different isolates of *C. gloeosporioides* Lal (*l.c.*) noticed much variation. According to him fructose and galactose were formed by *Artocarpus* and *Manihot* isolates, galactose by *Mussaenda* isolate, melibiose by *Annona* isolate and melibiose and galactose by *Codiaeum* isolate.

Isolate-B synthesised an oligosaccharide during the utilization of raffinose, but other isolates failed to do so. In this respect the behaviour of this isolate was similar to that of *Colletotrichum papayae* (Ghosh *et al.*, *l.c.*). No oligosaccharide was formed by various isolates of *C. gloeosporioides* studied by Ghosh *et al.* (*l.c.*), Chandra (*l.c.*), Prasad (*l.c.*), Ghosh (*l.c.*) and Lal (*l.c.*).

When the organisms were grown on a mixture of glucose-fructose-galactose, it was observed that in all cases glucose was used up earlier than galactose and fructose. Fructose and galactose were not consumed upto the end of the incubation period. The dry weights of all the isolates, except isolate-B, continued to increase upto the end of the incubation period on raffinose as well as on mixture of its hydrolytic products. Isolate-B showed slight decrease in dry weight on raffinose medium after 10 days, but the behaviour of this isolate was similar to others on mixtures of glucose, galactose and fructose. Isolate-B showed better growth on glucose-fructose-galactose mixture than on raffinose. In this respect the above isolate was like *Annona* and *Codiaeum* isolates of *C. gloeosporioides* (Lal, *l.c.*). The results obtained with the isolates A and C of *C. capsici* were similar to those of *Mussaenda* and *Manihot* isolates of *C. gloeosporioides* studied by Lal (*l.c.*)

as the growth was inferior on a mixture of glucose, fructose and galactose an on raffinose.

The variations between different isolates indicate the need of proper study of each form. Sterilization of the media containing these oligosaccharides did not show any breakdown product but the appearance of monosaccharides in such cases during the growth of different organisms appears to be due to enzymatic activity of the organism. It was also confirmed that pathogens which could assimilate a given oligosaccharide were also capable of using its hydrolytic products.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. D. D. Pant, Head of the Botany Department, for providing the laboratory facilities. They are also thankful to State C.S.I.R. (U.P.) for the financial help to one of them (B. Lal).

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