UTILIZATION AND SYNTHESIS OF OLIGOSACCHARIDES BY SOME PATHOGENIC ISOLATES OF *COLLETOTRICHUM GLOEOSPORIOIDES* PENZ.

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The paper includes the chromatographic studies on utilization of four oligosaccharides (viz. maltose, sucrose, lactose and raffinose) as well as mixtures of their hydrolytic products by five isolates of *Colletotrichum gloeosporioides* Penz. obtained from leaf-spots of *Artocarpus heterophylla* Lam. (Isolate A), *Annona squamosa* L. (Isolate B), *Mussaenda frondosa* Linn. (Isolate C), *Codiaeum variegatum* Blume (Isolate D) and *Manihot esculenta* Crantz. (Isolate E). Sucrose, maltose and raffinose were used up through hydrolytic pathway. Only in one case (Isolate E) hydrolytic product of lactose could be traced. The organisms were also capable of using the hydrolytic products of oligosaccharides. All the isolates synthesized one/two oligosaccharide(s) during the utilization of sucrose and maltose respectively. They failed to do so during the assimilation of lactose and raffinose. Growth of all the pathogens was better on sucrose than on a mixture of glucose and fructose. They exhibited inferior growth on maltose than on glucose. Isolates A, B and E showed superior growth on glucose-galactose mixture than on lactose; isolate C produced better growth on lactose than on its hydrolytic products, while isolate D revealed on special preference. When grown on mixture of glucose-fructose-galactose and raffinose, two isolates (B and D) showed better growth, and the remaining isolates exhibited inferior growth on the mixture than on raffinose.

INTRODUCTION

The oligosaccharides are complex sugars composed of two or more monosaccharides linked together by glycosidic bonds. These sugars are water soluble and on hydrolysis they yield monosaccharides. When a hydroxyl group of one monosaccharide unites with the reducing group of another a disaccharide is formed. In a similar manner tri-, tetra- and higher saccharides are formed by condensation. The glycosidic linkage of the oligosaccharide must be broken in order that the oligosaccharide may be available for metabolic transformation. Fruton and Simmonds (1958) have illustrated that in a biological system this cleavage is brought about with the help of enzymes by two general mechanism. The first involves the hydrolysis and the second the phosphorolysis of a glycosidic bond.

A large number of fungi including *Aspergillus niger* (Bacon and Bell 1953), *Penicillium spinulosum* (Bealing and Bacon 1953), *Aspergillus* spp.

(Aso and Shibasaki 1953), *Penicillium chrysogenum* (Giri *et al.* 1953), *Aspergillus flavus* (Giri *et al.* 1954), *Saccharomyces fragilis* (Robert and McFarren 1953), *Myrothecium verrucaria* (Cook and Stone 1953), *Phyllosticta* spp. (Bilgrami and Tandon 1957), *Pestalotia banksiana* and *P. citri* (Tandon and Bilgrami 1958), *Aspergillus* spp. (Mehrotra and Agnihotri 1961), *Cercosporina ricinella*, *Colletotrichum gloeosporiodes* and *Curvularia penniseti* (Tandon and Chandra 1962), *Alternaria tenuis*, *Colletotrichum capsici* and *C. gloeosporioides* (Chaturvedi 1961), *Macrophomina phaseoli*, *Fusarium solani* and *Botryodiplodia ananassae* (Bhargava 1962), *Colletotrichum gloeosporioides*, *Pestalotia pauciseta* and *Botryodiplodia theobromae* (Prasad 1963), *Colletotrichum gloeosporioides* and *C. dematium* (Ghosh 1964), *Trichothecium roseum*, *Curvularia lycopersici* and *Fusarium semitectum* (Kakkar 1964), *Colletotrichum gloeosporioides*, *C. papayae*, *Gloeosporium psidii* and *G. musarum* (Ghosh *et al.* 1965) and *Botryodiplodia theobromae* (Srivastava 1966) are known to synthesize new oligosaccharides when cultured on media with di- or trisaccharides. Recent researches have suggested that the so-called hydrolytic enzymes which cause the hydrolysis of the oligosaccharides can also cause the replacement reaction of the glycosidic bond resulting in the formation of a new oligosaccharide.

In nature, the oligosaccharides exist either as such or as the unit of polysaccharides. Occasionally these oligosaccharides are formed on partial hydrolysis of polysaccharides. They are, therefore, quite important in the nutrition of fungi. The present investigation was undertaken in order to determine (1) the ability of five isolates of *C. gloeosporioides* to utilize the oligosaccharides, (2) their pathway of utilization (indirect or direct) as well as (3) the probable effect of hydrolytic products on growth.

Materials and Methods

Single spore cultures of *Colletotrichum gloeosporioides* Penz. isolated from leaf-spots of *Artocarpus heterophylla* (Isolate A), *Annona squamosa* (Isolate B), *Mussaenda frondosa* (Isolate C), *Codiaeum variegatum* (Isolate D) and *Manihot esculenta* (Isolate E) 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 various oligosaccharides (viz. maltose, sucrose, lactose and raffinose) and the mixture of their hydrolytic products they were suitably substituted for glucose and their quantity was so adjusted as to furnish 4 g of carbon per litre. The most suitable *p*H for isolates B, C and E was found to be 6·0 while for A and D it was 5·0 and 5·5 respectively. The *p*H of media for subsequent studies was adjusted to the most suitable level in each case. Other methods used were similar to those mentioned by Lal and Tandon (1968).

Observations and Discussion

The data have been summarized in Tables I–IV. Regarding the utilization of oligosaccharides by fungi two theories have been suggested. According to Emil Fischer, the oligosaccharides are first hydrolysed with the help of enzymes into their component sugars and are then assimilated. Thus the failure to utilize an oligosaccharide may be due to the inability of the organism to synthesize the necessary hydrolytic enzymes. According to Richard Willstatter, oligosaccharides are utilized by a pathway that does not require hydrolysis before utilization (*see* Lilly and Bernett 1953). The results of the present study support the first view as all the sugars were utilized through indirect pathway.

Assimilation of sucrose (Table I)

Sucrose was readily utilized by all the fungi through the hydrolytic pathway and in each case the hydrolytic product glucose was used up earlier than fructose. A similar result was obtained when the pathogens were allowed to grow on mixture of glucose and fructose. All of them synthesized two oligosaccharides in the media containing sucrose. A simultaneous synthesis of oligosaccharides, along with the hydrolytic products in the medium containing this sugar, has also been reported by several authors. *Colletotrichum inamdarii* (Hasija 1965), however, failed to produce any synthetic oligosaccharide on sucrose.

The dry weights of all the pathogens on sucrose were maximum on 11th day, after which they decreased. They showed continuous increase in dry weight up to the end of the incubation period on mixture of glucose-fructose. Final dry weights of all the isolates were inferior on glucose-fructose mixture than on sucrose. Bhargava (1962) observed that dry weights of *Macrophomina phaseoli* and *Botryodiplodia ananassae* on glucose-fructose mixture and sucrose were similar, though the final dry weight of *Fusarium solani* was inferior on glucose-fructose mixture than on sucrose alone. According to Lilly and Barnett (1951), 'A complex carbohydrate and its hydrolytic products are not necessarily equivalent in all respects.'

Assimilation of lactose (Table II)

The rate of utilization of lactose was very slow, as all the isolates were unable to finish it even at the end of the incubation period of 15 days. None of them synthesized any oligosaccharide. Similar results were obtained for *Colletotrichum gloeosporioides* (Tandon and Chandra, Ghosh *et al.*), *C. capsici* (Chaturvedi) and *C. papayae* (Ghosh *et al.*). On the other hand, *C. gloeosporioides* (Prasad) and *C. inamdarii* (Hasija) utilized lactose in 14 and 12 days respectively. Only isolate E could partially hydrolyse lactose after 8 days and galactose was observed in the medium for 3 days. Srivastava

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. gloeosporioides

Organisms	Sugar	5 days		10 days		15 days		Presence of sugars (days)				
		Dry weight	Final *p*H	Dry weight	Final *p*H	Dry weight	Final *p*H	sucrose (Rf. 0·41)	glucose (Rf. 0·59)	fructose (Rf. 0·63)	oligo-saccharide I (Rf. 0·35)	oligo-saccharide II (Rf. 0·26)
Isolate A ..	sucrose	54·0	6·7	126·4	7·0	103·0	7·6	0–3	1–4	1–7	2–6	3–4
	½ glucose + ½ fructose	50·5	6·1	70·0	6·4	88·3	7·0	–	0–5	0–15	–	–
Isolate B ..	sucrose	49·9	6·7	143·0	7·0	101·9	7·3	0–5	3–6	3–8	2–10	3–7
	½ glucose + ½ fructose	15·5	5·5	50·0	6·1	61·0	7·3	–	0–10	0–15	–	–
Isolate C ..	sucrose	40·4	5·8	111·2	7·0	88·4	7·6	0–5	3–6	3–8	2–7	3–6
	½ glucose + ½ fructose	27·0	6·1	67·5	6·4	82·0	7·3	–	0–9	0–15	–	–
Isolate D ..	sucrose	30·0	6·7	115·0	6·7	89·6	7·3	0–4	1–6	1–8	1–6	2–6
	½ glucose + ½ fructose	22·5	5·5	55·0	6·1	60·6	6·4	–	0–8	0–14	–	–
Isolate E ..	sucrose	41·4	7·0	102·7	7·0	87·6	8·0	0–3	2–5	2–7	2–5	3–5
	½ glucose + ½ fructose	33·0	6·1	81·3	7·3	85·5	6·7	–	0–5	0–15	–	–

TABLE II

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

Organisms	Sugar	5 days		10 days		15 days		Presence of sugars (days)		
		Dry weight	Final *p*H	Dry weight	Final *p*H	Dry weight	Final *p*H	lactose (Rf. 0·43)	glucose (Rf. 0·61)	galactose (Rf. 0·57)
Isolate A	lactose	6·6	5·8	15·0	5·8	17·2	6·1	0–15	–	–
	½ glucose + ½ galactose	48·0	6·7	92·0	7·0	98·5	7·0	–	0–3	0–13
Isolate B	lactose	6·0	5·8	11·8	6·1	22·8	6·7	0–15	–	–
	½ glucose + ½ galactose	18·0	5·8	45·6	6·4	66·0	7·0	–	0–7	0–10
Isolate C	lactose	15·3	6·1	75·9	6·4	75·2	7·0	0–15	–	–
	½ glucose + ½ galactose	28·3	6·1	44·6	6·4	57·6	7·0	–	0–7	0–15
Isolate D	lactose	12·3	5·8	20·0	6·1	50·8	6·4	0–15	–	–
	½ glucose + ½ galactose	22·6	5·5	45·6	6·4	51·3	6·7	–	0–4	0–14
Isolate E	lactose	8·7	5·8	18·5	6·1	26·1	6·7	0–15	–	8–10
	½ glucose + ½ galactose	36·3	6·1	50·3	6·7	73·3	7·3	–	0–9	0–14

(1965), while working with *Curvularia verruculosa* and *C. trifolii*, also detected galactose as the hydrolytic product of lactose in the medium. The other hydrolytic product (viz. glucose) was not detected in the medium. It may be due to slow breakdown of lactose and simultaneous utilization of glucose by the pathogen. This indicated that β-galactosidase responsible for splitting of lactose was produced by the organism. The remaining four isolates, however, failed to produce the hydrolytic products in sufficient quantity. It is possible that they might have been used up simultaneously. Kakkar observed that even when there was no trace of hydrolytic products (viz. glucose and galactose) *Curvularia lycopersici* synthesized an oligosaccharide on lactose medium. Srivastava (1965) also reported oligosaccharide synthesis on lactose by *Curvularia verruculosa, C. pallescence* and *C. fallax.*

When grown on a mixture of glucose-galactose, all the organisms under study consumed glucose earlier than galactose, and their dry weight yields showed an increase up to the end of the incubation period. Except for *C. gloeosporioides* isolates C and D, the dry weights were superior on a mixture of glucose-galactose than on lactose. Mehrotra and Kumar (1962) obtained similar results with *Penicillium* spp. The difference was negligible in isolate D, being similar to three isolates of *C. gloeosporioides* and two isolates of *C. dematium* studied by Ghosh. Isolate C differed from the others in that the dry weight was greater on lactose than on the mixture of glucose and galactose.

Assimilation of maltose (Table III)

Maltose was used up by all the isolates through hydrolytic pathway. Although glucose, the hydrolytic product, could be detected only in the medium used by isolates C and E, an oligosaccharide was synthesized in every case which was an evidence of 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 *Colletotrichum.*

Chaturvedi, Tandon and Chandra as well as Prasad have also reported synthesis of an oligosaccharide by their isolates of *C. gloeosporioides* in maltose medium. Other isolates of the same species studied by Ghosh and Ghosh *et al.*, however, synthesized two oligosaccharides. *Colletotrichum capsici* (Chaturvedi) and *C. inamdarii* (Hasija) were altogether different in this respect as they did not show the formation of any oligosaccharide in maltose medium.

After 10 days of incubation, only isolate B showed a slight increase in dry weight, whereas the remaining four isolates exhibited a decrease. All the isolates grew better on glucose (hydrolytic product) than on maltose. Tandon and Chandra obtained similar results with *Colletotrichum gloeosporioides* and *Curvularia penneseti,* but they observed approximately similar growth of

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. gloeosporioides

Organisms	Sugar	5 days		10 days		15 days		Presence of sugars (days)		
		Dry weight	Final *p*H	Dry weight	Final *p*H	Dry weight	Final *p*H	maltose (Rf. 0·40)	glucose (Rf. 0·59)	oligo-saccharide I (Rf. 0·28)
Isolate A	maltose	30·4	6·1	91·6	6·7	74·0	7·3	0–10	–	5–10
	glucose	40·4	6·1	110·4	6·4	98·8	7·3	–	0–8	–
Isolate B	maltose	23·0	6·1	52·9	6·7	62·0	7·3	0–10	–	4–10
	glucose	52·8	6·7	122·5	7·0	110·0	7·3	–	0–8	–
Isolate C	maltose	54·1	6·1	99·6	6·7	78·2	7·3	0–6	3–7	3–6
	glucose	67·6	7·0	122·4	7·0	116·0	7·3	–	0–7	–
Isolate D	maltose	40·5	6·1	71·0	6·7	57·4	7·3	0–10	–	4–10
	glucose	42·1	6·1	109·1	6·7	99·6	7·3	–	0–8	–
Isolate E	maltose	37·0	6·1	71·8	6·7	56·9	7·3	0–9	4–10	3–10
	glucose	58·4	6·7	116·4	7·0	106·4	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. gloeosporioides

Organisms	Sugar	5 days		10 days		15 days		Presence of sugars (days)				
		Dry weight	Final *p*H	Dry weight	Final *p*H	Dry weight	Final *p*H	raffinose (Rf. 0·31)	melibiose (Rf. 0·35)	fructose (Rf. 0·65)	galactose (Rf. 0·57)	glucose (Rf. 0·61)
Isolate A ..	raffinose	28·8	5·8	45·5	6·1	76·6	7·3	0–9	–	3–11	3–13	–
	⅓ glucose + ⅓ fructose + ⅓ galactose	24·0	6·1	84·6	7·0	74·5	7·0	–	–	0–10	0–10	0–4
Isolate B ..	raffinose	8·0	5·8	15·8	6·7	22·5	7·3	0–15	9–15	–	–	–
	⅓ glucose + ⅓ fructose + ⅓ galactose	20·0	5·8	50·6	6·1	60·5	6·4	–	–	0–15	0–15	0–7
Isolate C ..	raffinose	23·0	5·8	35·5	6·1	75·0	7·0	0–12	–	–	4–13	–
	⅓ glucose + ⅓ fructose + ⅓ galactose	18·3	6·1	60·5	6·4	66·5	7·0	–	–	0–15	0–15	0–7
Isolate D ..	raffinose	16·3	5·8	29·0	6·1	59·0	6·7	0–15	5–14	–	4–15	–
	⅓ glucose + ⅓ fructose + ⅓ galactose	15·0	5·8	59·5	6·7	67·3	7·0	–	–	0–7	0–15	0–5
Isolate E ..	raffinose	29·9	6·1	47·8	6·1	52·8	6·7	0–14	–	5–13	5–14	–
	⅓ glucose + ⅓ fructose + ⅓ galactose	10·3	6·1	32·0	6·4	44·0	7·0	–	–	0–15	0–15	0–8

Cercosporina ricinella on media containing glucose/maltose. Mehrotra and Agnihotri working with five species of *Aspergillus* noticed that the growth was better on maltose than on glucose.

Assimilation of raffinose (Table IV)

The different isolates of the same species showed marked variation in the utilization of raffinose. Two isolates (B and D) could not consume it even in 15 days, while isolates A, C and E finished it in 9, 12 and 14 days respectively. Only one hydrolytic product, galactose, was detected in the medium used by isolate C, while fructose and galactose were detected in medium used by isolates A and E. Melibiose was detected in isolate B only. Isolate D developed both melibiose and galactose and in this respect it was like *C. gloeosporioides* studied by Ghosh *et al.* The presence of melibiose in the medium indicated that splitting of raffinose by isolates B and D took place at the β-linkage and enzyme β-glycosidase was produced. In other cases actual pathway of breakdown of raffinose could not be established.

During the utilization of raffinose by the isolates of *Colletotrichum* all the specific products of hydrolysis were not always detected, although the presence of some product(s) in the medium indicated that hydrolysis had taken place. The failure of the hydrolytic products to appear in the medium has been attributed to their simultaneous utilization. Bhargava and Prasad, working with *Fusarium solani* and *Colletotrichum gloeosporioides* respectively, reported that raffinose was utilized directly by these fungi since none of its hydrolytic products could be detected in the medium.

None of the isolates could synthesize oligosaccharide during the utilization of raffinose. In this respect their behaviour was similar to the isolate of the same species studied by Chandra, Prasad and Ghosh. *Colletotrichum papayae* (Ghosh *et al.*) was different as it synthesized an oligosaccharide in the medium during the utilization of raffinose.

When the organisms were grown on a mixture of glucose, galactose and fructose, it was observed that in every case glucose was used up earlier than galactose and fructose. Fructose was consumed earlier than galactose by isolate D, while isolate A utilized fructose and galactose simultaneously in 10 days. The remaining isolates failed to consume fructose and galactose up to the end of the incubation period of 15 days.

The dry weights of all the pathogens under study continued to increase up to the end of the incubation period. When the pathogens were grown on a mixture of glucose, galactose and fructose, two isolates (B and D) showed better growth on it than on raffinose. In this respect they were like *Nephalium* isolate of *Colletotrichum gloeosporioides* (Prasad). Three remaining isolates exhibited inferior dry weights on a mixture of glucose, galactose and fructose than on raffinose.

Conclusion

It is evident from the above results that different isolates of the same species showed marked variation in the utilization of various oligosaccharides as well as their component sugars. 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 various isolates appears to be due to enzymatic activity of these fungi. It was also confirmed that organisms which could assimilate a given oligosaccharide were also capable of using its hydrolytic products.

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