

THE UTILIZATION AND SYNTHESIS OF OLIGOSACCHARIDES BY TWO SPECIES OF *PESTALOTIA*

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ABSTRACT

The utilization of oligosaccharides by two species of *Pestalotia* viz., *P. banksiana* Cavara (isolated from the diseased leaves of *Grevelia robusta*) and *P. citri* Mundkur and Keshwala (isolated from the diseased leaves of *Citrus grandis*) was studied. Chromatographic technique was used to detect the presence of various sugars formed during assimilation. Three sources of nitrogen viz., ammonium chloride, asparagin and potassium nitrate were separately supplied in combination with different oligosaccharides.

Raffinose, sucrose and maltose were used after hydrolysis. Only two sugars viz., melibiose and laevulose were obtained during the assimilation of raffinose. The laevulose fraction was utilized faster while the melibiose fraction persisted upto the 15th day.

Sucrose was a good source. Chromatographic results showed that its both the components viz., glucose and fructose were utilized by these fungi. *Pestalotia citri* assimilated glucose earlier than fructose. *P. banksiana* also behaved in the same manner when asparagin or NH_4Cl were used as nitrogen sources, but with potassium nitrate the assimilation of both glucose and fructose was almost simultaneous. *P. banksiana* synthesized an oligosaccharide (Rf 0.51) on sucrose-asparagin medium.

Maltose was the best sugar for both the organisms. These fungi converted maltose by transglucosidation into an oligosaccharide (malto-triose) with simultaneous liberation of glucose.

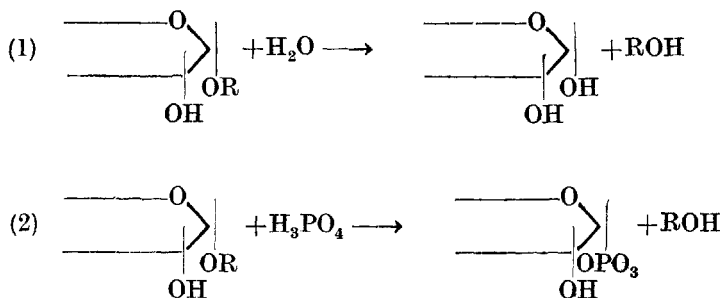
Cellobiose, lactose and melibiose were poorly utilized through a nonhydrolytic pathway. Melibiose (which is a component sugar of raffinose) influences the assimilation of raffinose.

Both the organisms preferred the ammonium nitrogen (NH_4Cl), which was followed by asparagin and KNO_3 respectively. It was observed that with certain exceptions the oligosaccharides or their hydrolytic products were assimilated slightly earlier if ammonium chloride was used as a source of nitrogen.

A combination of maltose- NH_4Cl was best for both the organisms.

Oligosaccharides are complex sugars composed of two or more monosaccharide units linked together through a glycosidic linkage. Fungi generally utilize these complex sugars after the glycosidic linkage is broken and free monosaccharides are available in the medium. Two general mechanisms effect the cleavage of the glycosidic linkage in a biological system. The first involves the hydrolysis (1) and the second one involves the phosphorolysis (2) of the glycosidic bond. Both these reactions are catalysed by specific enzymes. Recent investigations of various investigators have shown that the pathways of assimilation of different oligosaccharides vary with the organism. Their results have also shown that the so-called "hydrolytic enzymes" which cause the hydrolysis of oligosaccharides (during the course of its assimilation by fungi) can also cause the replacement reaction of the glycosidic bond under suitable experimental conditions. They observed that the oligosaccharides were synthesized as intermediates during the enzymic hydrolysis of disaccharides by fungi. Our recent investigations with another

species of *Pestalotia* viz., *P. mangiferae* have also shown that the assimilation of sugars was also influenced by the type of the nitrogen source available in the



medium. It was, therefore, considered desirable to examine the effect of association of different nitrogen sources with various oligosaccharides.

MATERIALS AND METHODS

The diseased leaves of *Grevelia robusta* and *Citrus grandis* were repeatedly observed at various places at Allahabad. They were infected with *Pestalotia banksiana* and *P. citri* respectively. The two organisms were isolated from the respective hosts. Six oligosaccharides viz., (raffinose¹, sucrose¹, maltose¹, lactose², melibiose³ and cellobiose¹), and three nitrogen sources (viz., ammonium chloride¹, asparagin² and potassium nitrate² were individually added to the basal medium (KH₂PO₄ 1.75 gms, MgSO₄, 7H₂O 0.75 gms. and double distilled water 1 litre) at a rate that supplied 4000 mgs. of carbon and 490 mgs. of nitrogen per litre respectively. Eighteen different combinations were prepared. Very thoroughly cleaned Pyrex glass ware were used. Equal quantities (25 ml.) of medium was dispensed into 150 ml. Erlenmeyer flasks. The media were fractionally sterilized for three consecutive days. Both the species of *Pestalotia* were daily inoculated at a fixed time for 15 days.

Three replicates were taken in each case. On the sixteenth day (when 1-15 day old cultures were available from different sets), the fungal mats from each set were separately filtered on Whatman No. 42 filter papers. The filtrate of each day was set aside for chromatographic analysis. The mycelial growth of sixth, eleventh and sixteenth days were dried and those dry weights served as quantitative measure of growth. The circular paper chromatographic technique (Ranjan *et al.*, 1955) was employed to detect the presence of various sugars formed during the assimilation of different oligosaccharides. n-butanol-pyridine-water (60 : 40 : 30) or n-butanol-acetic acid-water (4 : 1 : 5) were used as solvents and aniline-diphenylamine-phosphoric acid (Buchan and Savage, 1952) was used as spray reagent. The average R_f values of various sugars (with BAH as solvent) are given at appropriate places in the text and in Table 3.

1 B.D.H.
2 E. Merck.
3 DIFCO.

EXPERIMENTAL

Table 1 showing the dry weight (in mgs.) of *Pestalotia banksiana* obtained on 6th, 11th and 16th days on different combinations of oligosaccharides and nitrogen sources.

TABLE 1

Oligosaccharides

Nitrogen Source	Days of incubation	Raffinose	Sucrose	Maltose	Cellobiose	Lactose	Melibiose
Amm. chloride	5	30	48	60	10	20	8
	10	52	86	106	22	42	15
	15	64	108	144	28	54	20
Asparagin	5	22	60	56	8	20	7
	10	40	106	98	18	38	12
	15	52	132	126	24	50	14
Potassium nitrate	5	16	40	48	8	12	6
	10	28	68	84	14	21	10
	15	38	86	102	19	30	12

Table 1 shows that *P. banksiana* attained best growth on a maltose— NH_4Cl medium. The table also shows that with the exception of sucrose all other oligosaccharides were assimilated better when the ammonium nitrogen was available in the medium. Asparagin was, however, most suitable with sucrose though organic nitrogen (supplied by asparagin) was the next choice in all other combinations used in the present investigations. Nitrate nitrogen was not very suitable. The results also indicated that maltose and sucrose were best oligosaccharides. Raffinose and lactose could also support satisfactory growth when supplied with NH_4Cl or asparagin. Cellobiose and melibiose were definitely very poor oligosaccharides.

Table 2 showing the dry weight (in mgs.) of *P. citri* on 6th, 11th and 16th days on different combinations of various oligosaccharides and nitrogen sources.

TABLE 2

Oligosaccharides

Nitrogen Source	Days of incubation	Raffinose	Sucrose	Maltose	Cellobiose	Lactose	Melibiose
Amm. chloride	5	11	30	38	4	20	4
	10	26	52	66	7	32	8
	15	34	69	84	9	41	10
Asparagin	5	12	26	8	6	10	6
	10	23	44	56	11	17	12
	15	30	57	78	15	22	17
Potassium nitrate	5	4	24	30	2	8	3
	10	17	42	48	4	14	5
	15	24	50	64	7	17	8

It is obvious from Table 2 that in general the behaviour of *Pestalotia citri* was similar to that of *P. banksiana* but cellobiose and melibiose supported slightly

TABLE 3

Original oligosaccharide	Hydrolytic products	Synthetic products	<i>P. banketiana</i>				<i>P. citri</i>			
			Nitrogen source		Nitrogen source		Nitrogen source		Nitrogen source	
			NH ₄ Cl	Asparagin	KNO ₃	Asparagin	KNO ₃	NH ₄ Cl	Asparagin	KNO ₃
Raffinose (Rf) 0.40			1st— 6th day	1st— 6th day	1st— 6th day	1st— 10th day	1st— 6th day	1st— 9th day		
	Melibiose (Rf) 0.44		3rd— 15th "	5th— 15th "	5th— 15th "	2nd— 15th "	4th— 15th "	7th— 15th "		
	Fructose (Rf) 0.70		3rd— 6th "	3rd— 8th "	4th— 10th "	5th— 9th "	4th— 11th "	7th— 13th "		
Sucrose (Rf) 0.63			1st— 5th "	1st— 4th "	1st— 10th "	1st— 4th "	1st— 6th "	1st— 8th "		
	Glucose (Rf) 0.67		2nd— 7th "	2nd— 6th "	2nd— 12th "	2nd— 6th "	2nd— 10th "	2nd— 11th "		
	Fructose (Rf) 0.70		2nd— 9th "	2nd— 9th "	2nd— 12th "	2nd— 8th "	2nd— 11th "	2nd— 13th "		
Maltose (Rf) 0.57		Oligosaccharide I (Rf 0.49)		3rd & 4th "						
			1st— 5th "	1st— 5th "	1st— 6th "	1st— 10th "	1st— 10th "	1st— 12th "		
	Glucose (Rf) 0.67		3rd— 7th "	3rd— 8th "	2nd— 10th "	3rd— 8th "	7th— 10th "	4th— 14th "		
Cellobiose (Rf) 0.57 Lactose (Rf) 0.53 Melibiose (Rf) 0.44		Maltotriose (Rf 0.22)	4th— 5th "	4th— 7th "	4th— 7th "	5th— 7th "	5th— 10th "	7th— 11th "		
			1st— 13th "	1st— 13th "	1st— 15th "	1st— 15th "	1st— 15th "	1st— 15th "		
			1st— 12th "	1st— 10th "	1st— 13th "	1st— 11th "	1st— 15th "	1st— 15th "	1st— 15th "	

better growth when they were used in combination with asparagin than with ammonium chloride. The nitrate nitrogen was comparatively poorer than ammonium or organic nitrogen.

The average Rf values of various sugars and the results of the chromatographic investigations have been summarized in Table 3.

Table 3 showing the reaction of various oligosaccharides as well as the time of appearance of the hydrolytic products and their rate of utilization by *P. banksiana* and *P. citri*.

Table 3 shows that raffinose was utilized through a hydrolytic pathway by both the species of *Pestalotia*. Only two sugars were detected in the breakdown of this oligosaccharide viz., melibiose (Rf. 0.44) and fructose (Rf 0.70). Melibiose fraction could not be consumed completely by any of the two organisms, while the laevulose fraction was utilized. The rate of assimilation of laevulose greatly depended on the type of the nitrogen available in the substrate. Sucrose was also used after hydrolysis. Generally, both the organisms assimilated glucose earlier than fructose. In the presence of potassium nitrate *P. banksiana* utilized both the hydrolytic products (viz., glucose and fructose), simultaneously. This organism was also capable of synthesizing an oligosaccharide (Rf 0.49) with sucrose in presence of asparagin. Maltose was hydrolysed to glucose before assimilation. A simultaneous synthesis of maltotriose Rf. 0.22 (an oligosaccharide) was also observed during the utilization of this sugar. In every case this synthetic product appeared as an intermediate and was not dependent on the source of nitrogen. The above table also shows that the remaining oligosaccharides (viz., lactose, melibiose and cellobiose) were utilized through a non-hydrolytic pathway. Except lactose in combination with ammonium chloride, *P. citri* could not finish any of these three oligosaccharides even in 15 days. *P. banksiana* was slightly faster in consuming these oligosaccharides, though cellobiose and melibiose were not used up completely when supplied in combination with potassium nitrate.

DISCUSSION

Neuberg and Mandl (1950) reported that invertase* hydrolyses raffinose into fructose and melibiose only, though normally it can be hydrolysed into 5 products (viz., sucrose, melibiose, glucose, fructose and galactose). In the present investigations only laevulose and melibiose have been noticed and thus it appears that invertase is secreted by both the species of *Pestalotia*. This enzymic system also appears to function during the assimilation of sucrose, as both glucose and fructose were found to be present. *P. banksiana* exhibited slightly different behaviour on a sucrose-asparagin medium because besides the hydrolytic products of sucrose an oligosaccharide (Rf 0.49) was also synthesized in the medium on the 3rd and 4th days. Synthesis of a similar oligosaccharide from sucrose by *Aspergillus flavus* and *A. niger* has been reported by Giri *et al.* (1954) and Bacon and Bell (1953) respectively. Bealing and Bacon (1953) using enzymic extract of *Aspergillus niger* attributed the breakdown of sucrose to the transference of fructose residues to suitable acceptors by a β -fructofurnasidase. This interpretation explains the synthesis of an intermediate oligosaccharide during the hydrolysis of sucrose. Giri *et al.* (1954) who were able to separate this oligosaccharide have established that the oligosaccharide in question was a trisaccharide. They further found that on acid hydrolysis this oligosaccharide was hydrolysed to fructose and glucose which existed in the ratio of 2 : 1.

Oligosaccharide formation by transglycosidation with enzymes from *Aspergillus oryzae* has also been reported by Pazur (1954). Edelman and Bealing (1953) have

* It has also been termed as succharase, sucrase and β -fructosidase.

suggested that glycosidases (usually considered to be merely catalysts of glycosidic bonds), can also catalyse replacement reaction. There is considerable controversy in literature about the identity or non-identity of various enzymic materials. Recent researches of various biochemists suggest that the cleavage of maltose was not accomplished by a hydrolytic mechanism but by a transglycosidation leading to the formation of oligosaccharides. However, the discovery of these enzymes is extremely important as they demonstrate that the synthesis of the glycosidic bond in all living systems does not involve the direct participation of the phosphate.

The authors in their previous investigation (1958) had observed that the utilization of two identical oligosaccharides may be accomplished through two different pathways by a particular organism. The present results also confirmed the previous observations which showed that maltose and cellobiose (which are identical in structure except for the mode of linkage between two glucose units) were not only used through two different pathways, but their availability was totally different for both the species of *Pestalotia*. The former was found to be the best source of oligosaccharide while the latter was very poorly and slowly utilized. On the basis of present results it can also be interpreted that probably the fungi under study were incapable of synthesizing β -glucosidase and so they were unable to hydrolyse cellobiose (which has a β -glucosidic link).

The results also reveal that *P. citri* could attain only negligible growth on raffinose-KNO₃ or maltose-NH₄Cl media till the 6th day. A glance at Table 3 shows that the hydrolytic products of raffinose or maltose were not detected chromatographically till that day. This further showed that brisk mycelial growth started only after the production of the hydrolytic products.

None of the two organisms were able to utilize melibiose satisfactorily. Chromatographic results indicated that even the melibiose fraction formed during the assimilation of raffinose was not assimilated and it remained accumulated till the 15th day. The comparatively poor results with raffinose appear to be connected with the unsatisfactory utilization of this substance (melibiose). The present investigations also revealed that though lactose was assimilated by both the organisms, through a nonhydrolytic pathway, yet it was comparatively a better source than either cellobiose or melibiose, which were also used up through a non-hydrolytic pathway.

The present species of *Pestalotia* behaved like *P. mangiferae* (Bilgrami, 1956) as it was found that ammonium nitrogen was more suitable than nitrate nitrogen.

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