THE UTILIZATION OF MONOSACCHARIDES BY *PESTALOTIA BANKSIANA* AND *P. CITRI*

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ABSTRACT

The utilization of eight monosaccharides by *Pestalotia banksiana* and *P. citri* was studied in detail. The influenee of three different types of nitrogen (viz. ammonium, organic and nitrate) on the assimilation rate of different sugars was also investigated. Circular paper chromatographic method was employed for daily analysis of the medium.

Glucose was the best monosaccharide for the present organisms. Maximum dry weight was attained when this sugar was used in combination with ammonium chloride.

Mannose, galactose, arabinose and xylose were also satisfactory sources. Levulose was good for the growth of *P. banksiana* only.

Rhamnose and sorbose were very poor sources. Chromatographic analysis of the medium showed that these sugars were not consumed completely by any of the organisms even in 15 days.

Addition of sorbose had very pronounced inhibitary effect on other monosaccharides. Utilization of all other sugars was delayed in presence of sorbose.

Ammonium nitrogen was superior to organic or nitrate nitrogen.

Generally all the polysaccharides or oligosaccharides first get hydrolysed into simple soluble components before they can be utilized as food by the cells of the micro-organisms. The action of proper extracellular enzyme is necessary to bring about the reaction required to change the substance into more simple soluble compounds which can then diffuse through the cell membranes. Recent investigations of Tandon and Bilgrami (1958) have shown that two species of *Pestalotia* viz. *P. banksiana* and *P. citri* utilized some of the common oligosaccharides like raffinose, sucrose and maltose etc., after hydrolysis. As the monosaccharides were the ultimate sugars to be produced in the medium, it was decided to study the influence of 8 different monosaccharides on the rate and amount of growth of those two fungi.

Previous observations of the authors (Tandon and Bilgrami, 1957, 1958) had shown that the utilization of sugars by the species of *Pestalotia* was also dependent on the type of nitrogen source present in the substratum. In view of those results it was decided to supply three different types of nitrogen sources individually in combination with each of the monosaccharide used in the present investigation.

Influence of mixing sorbose (which has generally been reported to be very poor carbon source for most of the fungi) with other sources of carbon was also studied.

MATERIALS AND METHODS

Single spore cultures of *Pestalotia banksiana* and *P. citri* used in the earlier investigations were employed. Eight monosaccharides (viz., glucose, levulose, galactose, mannose, sorbose, arabinose, xylose and rhamnose) and three nitrogen sources viz., ammonium chloride, asparagin and potassium nitrate, were individually supplied to the culture medium*. It had 4000 mgs. of carbon and 490 mgs. of nitrogen per litre. Purest available chemicals of E. Merck or B.D.H. make were used. 25 ml. of liquid nutrient (contained in 150 ml. Erlenmeyer flasks)

*KH_2 Po_4–1·75 gms, $MgSo_4.7H_2o$. 0·75 gm and double distilled water 1 litre.

was used to culture the organisms. The various media were autoclaved at 15 lbs. pressure for 15 mts. Both the fungi were daily inoculated at a fixed time (± 25 mts) for 15 days in the autoclaved solutions.

Triplicate sets were used in every case. Fungal colonies from each set were separately filtered on the 16th day. Filtrate of each day was chromatographically analysed while dried fungal mycelium was used as a measure of growth. Circular paper chromatograms were used. Butanol-acetic acid-water (4 : 1 : 5) and aniline diphenyl amine phosphate served as solvent and spray reagent respectively. The average Rf values of various sugars were measured and have been recorded in the text.

TABLE I

Showing the dry weights (in mgs) of Pestalotia banksiana and P. citri obtained on 6th, 11th and 16th day on different combinations of monosaccharides and three sources of nitrogen.

Monosaccharides	Days of incubation	*P. banksiana*			*P. citri*		
		Nitrogen sources			Nitrogen sources		
		Amm. chloride	Asparagin	Potassium nitrate	Amm. chloride	Asparagin	Potassium nitrate
Glucose	5	40	35	32	31	27	26
	10	69	66	58	56	42	44
	15	96	84	76	69	59	58
Levulose	5	39	40	33	20	15	10
	10	75	61	57	31	27	18
	15	92	78	74	44	36	22
Mannose	5	30	32	21	26	20	21
	10	57	59	39	44	35	32
	15	76	70	50	54	42	38
Galactose	5	37	33	24	28	21	22
	10	66	58	43	41	39	37
	15	82	68	56	57	48	46
Sorbose	5	9	2	2	6	4	3
	10	16	11	6	18	13	5
	15	20	14	8	20	16	5
Arabinose	5	27	21	22	28	25	24
	10	44	36	32	46	44	37
	15	56	48	40	60	52	48
Xylose	5	33	29	25	18	15	13
	10	51	48	43	30	27	21
	15	68	60	54	40	33	28
Rhamnose	5	21	9	8	10	9	8
	10	26	19	16	19	16	15
	15	32	25	23	26	21	19

EXPERIMENTAL

It is evident from Table No. 1 that glucose in combination with NH_4Cl served as best monosaccharide for both the species of *Pestalotia*. *P. banksiana* exhibited almost similar growth on levulose also, while *P. citri* showed much less growth on this substance than on glucose. Mannose and galactose were also satisfactory

sources but they were decidedly inferior to glucose. Sorbose was the poorest monosaccharide. Amongst the pentoses arabinose was slightly superior to galactose or mannose for *P. citri*, while *P. banksiana* exhibited greater liking for xylose than for arabinose. Rhamnose was a poor source but it was better than sorbose for both the species of *Pestalotia*.

The influence of addition of sorbose on the utilization of other monosaccharides has been recorded in Table 2.

A comparison of Tables 1 and 2 shows that addition of sorbose to glucose, levulose, mannose or galactose or to any of the pentoses resulted in limiting the efficiency of these sugars also. It was observed that the vegetative growth of both the organisms on different sugars was reduced to less than half by the addition of sorbose.

TABLE II

Showing the influence of addition of Sorbose on the dry weight yield (in mgs.) of P. banksiana and P. citri.

Monosaccharides	No. of Days of incubation	*P. banksiana*			*P. citri*		
		Nitrogen sources			Nitrogen sources		
		NH_4Cl	Asparagin	Potassium nitrate	Amm. chloride	Asparagin	Potassium nitrate
Glucose	5	15	11	10	12	9	10
and	10	31	26	23	23	17	21
Sorbose	15	44	36	32	30	24	28
Levulose	5	14	10	8	11	8	9
and	10	32	23	21	19	18	16
Sorbose	15	45	34	30	28	24	22
Mannose	5	11	10	7	8	6	6
and	10	23	21	15	17	14	11
Sorbose	15	33	32	22	26	21	16
Galactose	5	13	9	8	5	5	6
and	10	24	20	17	11	12	10
Sorbose	15	34	28	26	18	14	13
Arabinose	5	8	7	5	9	8	6
and	10	18	16	12	20	15	13
Sorbose	15	27	25	18	29	20	19
Xylose	5	12	9	7	7	6	5
and	10	25	19	14	12	11	8
Sorbose	15	31	24	19	17	14	10
Rhamnose	5	8	5	5	6	4	5
and	10	13	11	8	10	9	8
Sorbose	15	15	13	9	13	11	8

The results of chromatographic analysis of different media (using NH_4Cl as nitrogen source) are summarized in Table 3.

TABLE III

Showing the results of the Chromatographic analysis of different media during the growth of P. banksiana *and* P. citri

Name of the monosaccharide	*P. banksiana*		*P. citri*	
	A	B	A	B
Sorbose Rf 0.72	15	15	15	15
Glucose Rf 0.65	7	10	10	13
Mannose Rf 0.69	10	13	12	15
Levulose Rf 0.70	8	10	11	15
Galactose Rf 0.64	8	11	14	15
Arabinose Rf 0.71	11	14	9	13
Xylose Rf 0.74	12	15	13	15
Rhamnose Rf 0.82	15	15	15	15

(Column A denotes the number of days taken in utilizing a single monosaccharide. Column B indicates the number of days when sorbose was used in combination with a particular monosaccharide).

Table 3 clearly shows that utilization of all the monosaccharides by both the species of *Pestalotia* was delayed when sorbose was mixed with them. Rhamnose was not consumed either singly or in combination with sorbose up to 15 days.

DISCUSSION

Monosaccharides are the most easily assimilable carbohydrates by micro-organisms. Our recent investigations have revealed that the complex carbohydrates which were usually present in the host plant, are first converted into simpler monosaccharides like glucose and levulose and then utilized. The results of the present investigation showed that glucose is the best monosaccharide. Several investigators have reported that this sugar is the most efficient source of carbon for a large number of fungi. Some have reported that its addition to other sugars had a stimulatory effect for some of the fungi investigated by them. Our earlier investigations had shown that maltose (a disaccharide, composed of two glucose units) was the best oligosaccharide for both *Pestalotia banksiana* and *P. citri*. A comparison with previous results (Tandon and Bilgrami 1958) shows that maltose is a much better source than glucose for both the species of *Pestalotia*. The superiority of maltose over glucose has also been reported by Brock (1951) and Agarwal (1955) for *Morchella esculenta* and *Curvularia penniseti* respectively. Our unpublished work showed that starch is also a comparatively better source than glucose. Blank and Talley (1941) also found starch and maltose to be better sources for *Phymatotrichum omnivorum*. They suggested that impurities of the chemicals might have been responsible for this behaviour. Maltose and starch of extra pure qualities were used by the authors and it is thus clear that the results are not due to any impurity. It appears that the behaviour of maltose or starch may be connected with the availability of *active glucose* during decomposition of those substances by *Pestalotia banksiana* and *P. citri*.

Chromatographic studies showed that with the exception of rhamnose and sorbose all other sugars are consumed by the present organisms within the incubation period. The results also showed that the addition of sorbose to the culture medium results in slow utilization of good sources also. Levulose, mannose, xylose and galactose, which were individually consumed by *P. citri* in 11, 12, 13 and 14 days respectively, were not consumed completely even in 15 days when used in association with sorbose. It was also clear that addition of sorbose to any of these

monosaccharides considerably reduces the dry weight. It was interesting to find that mannose lost its efficiency to such an extent that mycelial growth of *P. citri* on a mixture of mannose-sorbose medium was similar to that on sorbose alone. Lilly and Barnett (1953) have also reported the inhibitory effect of sorbose for a number of fungi. They reported that inhibitory action of sorbose is greater in presence of sucrose or maltose than with glucose. They further mentioned that in fact this sugar (sorbose) may be stimulatory in presence of glucose and inhibitory in presence of sucrose or maltose. The size and complexity of the sugar molecule has been suggested to be the possible reason for this behaviour. The present results, however, showed that addition of sorbose to other monosaccharides was not beneficial in any case. The reason for the toxic effect of sorbose on the growth of micro-organisms is not clear to any worker so far. Chromatographic analysis of the medium showed that sorbose was not utilized by any of the two species of *Pestalotia* up to 20 days though the autolysis of the fungal mycelium had already started by that time.

Lilly and Barnett (1953) recorded interesting results of the nitrogen source on the rapidity with which sorbose inhibition was overcome. They reported that hydrolysed casein counter acted sorbose inhibition more than asparagin or nitrate nitrogen. The results reported herein showed that ammonium nitrogen was most suitable source of nitrogen. It is felt that superiority of ammonium chloride is due to the specific choice of these fungi for the ammonium nitrogen and not on account of the fact that ammonium chloride prevents the inhibitory effect of sorbose. This statement is supported by the fact that these fungi as well as several other species of *Pestalotia* have been reported to exhibit greater choice for ammonium nitrogen than for nitrate or organic nitrogen.

Amongst pentoses rhamnose was inferior to arabinose or xylose. Rhamnose is a methyl pentose i.e. it has CH_3 grouping in its structure, while the other two pentoses do not have this group. It is possible that CH_3 group in rhamnose may have some prominent effect in the type of growth observed on this substance.

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