ASSIMILATION OF DISACCHARIDES BY SOME FUNGI CAUSING "LEAF SPOT" DISEASES

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Introduction

Assimilation of disaccharides by the micro-organisms generally depends upon the production of necessary hydrolytic enzymes. Non-utilization of these complex sugars is usually associated with lack of such enzymes. Instances are known where the complex sugars may not be utilized by an organism even though its hydrolytic products may support good growth. Thus Margolin (1942) found that *Syncephalastrum racemosum* could utilize the hydrolytic products of lactose (*viz.*, glucose and galactose), but it was incapable of using lactose. According to him the non-utilization of lactose was due to the inability of that organism to synthesize lactase. Mandels (1954), however, suspected that in some cases the disaccharides could be assimilated even without being hydrolysed. He suggested that the spores of the fungus *Myrothecium verrucaria* did not use sucrose through a hydrolytic pathway. Smith (1949) also obtained almost similar results with *Marasmius chordalis* and according to him, this organism attacked cellobiose by a route that involved neither preliminary hydrolysis nor phosphorylation. The observations of Smith (1949) and Mandels (1954) indicate the direct utilization of disaccharides by some fungi, but such records of direct assimilation of complex sugars are rare. Hawker and Chaudhari (1946) reported that the rate of inversion of sucrose determined the amount of growth of *Podospora* sp., *Pyronema confluens* and *Chætomium cochliodes*. It is thus evident that not only the pathway of utilization of disaccharides may vary with different fungi, but even their rate of assimilation may depend on the rate of hydrolysis of the sugar.

An attempt has, therefore, been made to undertake chromatographic studies to establish the pathway of utilization (hydrolytic or non-hydrolytic) of some common disaccharides (*viz.*, sucrose, maltose and lactose) by three leaf spot causing fungi.

Materials and Methods

The three fungi, *viz.*, *Phyllosticta cycadina* (Pass.), *Phyllosticta artocarpina* (Syd. *et* Butl.) and *Pestalotia mangiferæ* (Butl.), which were responsible

for the leaf spot diseases of *Cycas revoluta*, *Artocarpus heterophyllus* and *Mangifera indica* respectively, were isolated from the infected leaves of the above mentioned hosts. The methods of isolation, purification and subculturing were similar to those of Tandon and Bilgrami (1954). Asthana and Hawker's medium A* which has glucose as the only source of carbon was selected as the basal medium. Glucose was replaced by different disaccharides and mixture of their hydrolytic products. Care was taken to include the same amount of carbon (*i.e.*, 2 gm. per 1,000 ml. in each case). Pyrex glass wares and purest available chemicals were used. Fixed quantity of medium (*viz.*, 25 ml.) was taken in each 150 ml. conical flasks which were fractionally sterilized by steaming for half an hour daily for three successive days. Solutions containing these sugars were daily inoculated for 15 days with different organisms by agar disc method of Garrett (1936). The fungal mat from each set was filtered separately on the sixteenth day when one to fifteen days old cultures were available. The calculated dry weight was used as the quantitative measure for daily growth. The filtrate from each set was chromatographically analysed to detect the presence of the sugars. The method described by Ranjan *et al.* (1955) was used for this purpose. A circular piece of Whatman filter-paper No. 1, diameter 27 cm. having 12 radial sectors separated by 12 radial cuts was used. Drops of known volume (0·005 ml.) were taken from the filtrate of each day and they were placed at the positions located for this purpose (just above the numbers 1, 2, 3, ... 10, etc.). Sometimes index solutions of known sugars were also kept on the same chromatogram because this could facilitate the identification and comparison of bands. After placing the drops, the chromatograms were run with *n*-butanol-acetic acid and water (4 : 1 : 5). For separation of glucose and galactose (which gave a common band with the above solvent) *n*-butanol-pyridine and water (60 : 40 : 30) were used as the developing solvent. The chromatograms had single paper wick (4×2 cm.) and were run for about 7 hours in the above solvent. They were then dried at room temperature for 2–3 hours and were subsequently sprayed with a mixture of aniline-diphenyl amine phosphate (5 volumes of 4% aniline, 5 volumes of 4% diphenyl amine and 1 volume of phosphoric acid). After spraying, the chromatograms were left at room temperature for 3–4 hours and were then placed in an electric oven at 110° C. for 90 seconds. The average R_f values of various sugars were determined and they have been recorded in brackets at appropriate places in the text.

* KNO_3 3·5 gm., dextrose 5·0 gm., KH_2PO_4 1·75 gm., $MgSO_4 \cdot 7H_2O$ 0·75 gm., distilled water 1 litre.

The daily dry weight of these organisms on different disaccharides has also been recorded and their growth has been compared with the growth obtained on a mixture of corresponding monosaccharides, which were formed by the hydrolysis of various disaccharides.

OBSERVATIONS

1. *Assimilation of Sucrose* (R_f 0·61).—Chromatographic analysis of all the three hosts showed the presence of this sugar as well as of its hydrophytic products (*viz.*, glucose and fructose). Most of the fungi hydrolyse this sugar before assimilation. Daily chromatographic analysis of the medium revealed that the present organisms also utilized sucrose through a hydrolytic pathway. The rate of conversion of sucrose, however, varied with the organisms. It was observed that between 2nd and 3rd day, the two species of *Phyllosticta* converted the entire sucrose into a mixture of glucose (R_f 0·66) and fructose (R_f 0·69) and within that period it (sucrose) totally lost its identity in the medium. The same chromatograms also established that both these fungi fully assimilated glucose, three days earlier than fructose. The hydrolysis rate of this sugar (*viz.*, sucrose) by *Pestalotia mangiferæ* was much slower because it could not be hydrolysed completely even upto 7th day. It was also noticed that both the hydrolytic products of sucrose were simultaneously consumed on the 15th day.

The daily dry weight growth rate of these fungi on sucrose as well as a mixture of its hydrolytic products is summarized in Table I.

It is evident from the table that growth rate of both the species of *Phyllosticta* increased upto 5th day but in each case there was a progressive fall from the 6th day. The dry weight of *P. cycadina* increased upto 15th day while that of *P. artocarpina* increased upto 16th day. *Pestalotia mangiferæ*, however, did not show any growth upto 3rd day. Chromatographic analysis of the medium had indicated that sucrose was not hydrolysed by *Pestalotia mangiferæ* upto that day (3rd day). Table I also shows that the growth rate of *Pestalotia mangiferæ* increased upto 6th day and then it decreased. The dry weight of the organism, however, continued to increase upto the 16th day. These organisms were also grown on a mixture of glucose and fructose and it was observed that all the three fungi exhibited almost similar behaviour on this mixture as on sucrose except for the fact that *Pestalotia mangiferæ* which did not show any growth on sucrose upto 3rd day was capable of producing mycelium from the 2nd day.

2. *Assimilation of Maltose* (R_f 0·55).—Maltose on hydrolysis yields glucose only. Chromatographic analysis of the medium containing this

TABLE I

Showing daily dry weight (in mgm.) and growth rate of Phyllosticta cycadina, P. artocarpina *and* Pestalotia mangiferæ *on sucrose as well as a mixture of its hydrolytic products*

	Days															
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Phyllosticta cycadina—																
1 Daily dry wt. on sucrose ..	0·0	3·3		27·2	40·0	50·5	59·5	67·7	75·0	81·5	84·5	86·8	88·0	88·5	88·8	88·8
2 Daily dry wt. on mixture of glucose and fructose	0·0	3·9	15·3	29·0	44·0	54·0	63·3	71·5	78·0	84·0	86·3	88·0	89·0	89·7	90·0	90·0
3 Growth rate on sucrose ..	0·0	3·3	11·7	12·2	12·8	10·5	9·0	8·2	7·3	6·5	3·0	2·3	1·2	0·5	0·3	..
4 Growth rate on mixture of glucose and fructose	0·0	3·9	11·4	13·7	15·0	10·0	9·3	8·2	6·5	6·0	2·3	1·7	1·0	0·7	0·3	..
Phyllosticta artocarpina—																
1 Daily dry wt. on sucrose ..	0·0	4·3	14·0	26·0	42·3	51·8	60·8	69·0	77·2	85·0	90·5	92·5	94·3	95·5	96·5	97·0
2 Daily dry wt. on mixture of glucose and fructose	0·0	3·4	12·5	25·1	41·5	52·0	60·2	68·0	75·5	82·3	85·5	88·5	90·8	92·8	94·0	94·5
3 Growth rate on sucrose ..	0·0	4·3	9·7	12·0	16·3	9·5	9·0	8·2	8·2	7·8	5·5	2·0	1·8	1·2	1·0	0·5
4 Growth rate on mixture of glucose and fructose	0·0	3·4	9·1	12·6	16·4	10·5	8·2	7·8	7·5	6·8	3·2	3·0	2·3	2·0	1·2	0·5
Pestalotia mangiferæ—																
1 Daily dry wt. on sucrose ..	0·0	0·0	0·0	4·6	9·5	17·0	23·1	28·0	32·5	36·5	40·1	43·5	46·8	49·8	52·7	55·5
2 Daily dry wt. on mixture of glucose and fructose	0·0	2·5	10·5	19·4	30·5	37·5	44·0	48·2	50·5	52·5	54·2	55·5	56·5	57·3	57·5	57·6
3 Growth rate on sucrose ..	0·0	0·0	0·0	4·6	4·9	7·5	6·1	4·9	4·5	4·0	3·6	3·4	3·3	3·0	2·9	2·8
4 Growth rate on mixture of glucose and fructose	0·0	2·5	8·0	8·9	11·1	7·0	6·5	4·2	2·3	2·0	1·7	1·3	1·0	0·8	0·2	0·1

sugar revealed the formation of glucose during its assimilation. The rate of hydrolysis of maltose also varied with the organism. Culture medium inoculated with *P. cycadina* showed the presence of glucose (R_f 0·66) on the 3rd day but it disappeared on the 8th day. Traces of maltose were also observed upto 8th day. In the medium on which *P. artocarpina* was growing, the formation of glucose was observed on the second day. In this case presence of glucose was recorded upto 9th day, while that of maltose upto 7th day only. The rate of hydrolysis of maltose by *Pestalotia mangiferæ* was very slow. Glucose appeared on the 6th day, and persisted upto 11th day while maltose was present only upto 10th day. During the process of assimilation of maltose by these fungi, it was observed that besides glucose one more sugar was also formed in the medium. The R_f value of this band (band III) was 0·18 which indicated it to be maltotriose (an oligosaccharide) and it was suspected that this sugar was synthesized during the hydrolysis of maltose. For further confirmation of this band the chromatogram was sent to Professor K. V. Giri* at Bangalore. Giri (1955) in a personal communication also agreed that the particular band (band III) was of maltotriose.†

The daily dry weight of these organisms on maltose and its hydrolytic product (glucose) is recorded in Table II.

Table II indicates that growth rate of both the species of *Phyllosticta* increased upto 5th day, while that of *Pestalotia mangiferæ* continued to increase upto 7th day. The dry weight of *Phyllosticta cycadina* and *Pestalotia mangiferæ* increased upto 15th day. *Phyllosticta artocarpina*, however, showed an increase in the dry weight upto 16th day. No growth of *Pestalotia mangiferæ* was evident upto 3rd day. This appears to be connected with delayed and slow hydrolysis of maltose by this fungus. A comparison of growth of these organisms on maltose with that of glucose showed that all the three organisms attained better mycelial growth on maltose than on glucose. Chromatographic analysis of the medium containing glucose only revealed that it was consumed in 9, 12, and 14 days by *Phyllosticta cycadina*, *P. artocarpina* and *Pestalotia mangiferæ* respectively.

3. *Assimilation of Lactose* (R_f 0·51).—Lactose has not been reported to be present in plants. Hydrolysis of lactose with acids or lactase yields a molecule each of glucose and galactose. Daily chromatographic analysis of the medium showed that this sugar was not hydrolysed by any of the three

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† In spite of best efforts, this sugar could not be obtained in synthetic form and it was, therefore, not possible to include it for detailed studies or to compare the band obtained with the band of maltotriose.

TABLE II

Showing daily dry weight (in mgm.) and growth rate of Phyllosticta cycadina, P. artocarpina *and* Pestalotia mangiferæ *on maltose as well as its hydrolytic product (glucose)*

	Days															
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Phyllosticta cycadina—																
1 Daily dry wt. on maltose ..	0·0	5·0	19·0	35·0	53·0	66·5	74·5	80·0	84·0	86·0	87·0	87·9	88·5	89·0	89·3	89·3
2 Daily dry wt. on glucose ..	0·0	3·3	13·5	26·0	39·2	53·0	63·5	69·5	74·3	78·5	82·7	83·3	83·8	84·1	84·2	84·2
3 Growth rate on maltose ..	0·0	5·0	14·0	16·0	18·0	13·5	8·0	5·5	4·0	2·0	1·0	0·9	0·6	0·5	0·3	..
4 Growth rate on glucose ..	0·0	3·3	10·2	12·5	13·2	13·8	10·5	6·0	4·8	4·2	4·2	0·6	0·5	0·3	0·1	..
Phyllosticta artocarpina—																
1 Daily dry wt. on maltose ..	0·0	4·5	17·0	32·0	52·0	63·0	73·0	81·5	87·5	92·5	97·0	100·0	102·5	104·5	105·5	106·0
2 Daily dry wt. on glucose ..	0·0	3·5	12·0	22·5	34·6	47·0	59·5	69·0	76·2	82·0	87·0	91·3	91·7	92·0	92·2	92·2
3 Growth rate on maltose ..	0·0	4·5	12·5	15·0	20·0	11·0	10·0	8·5	6·0	5·0	4·5	3·0	2·5	2·0	1·0	0·5
4 Growth rate on glucose ..	0·0	3·5	8·5	10·5	12·1	12·4	12·5	9·5	7·2	5·8	5·0	4·3	0·4	0·3	0·2	..
Pestalotia mangiferæ—																
1 Daily dry wt. on maltose ..	0·0	0·0	0·0	6·0	17·5	33·5	55·0	63·0	67·0	69·4	71·5	73·2	74·5	75·3	75·5	75·5
2 Daily dry wt. on glucose ..	0·0	2·4	8·6	15·1	22·2	30·0	38·5	42·5	46·5	50·0	53·3	56·0	58·2	60·2	62·2	63·5
3 Growth rate on maltose ..	0·0	0·0	0·0	6·0	11·5	16·0	21·5	8·0	4·0	2·4	2·1	1·7	1·3	0·8	0·2	..
4 Growth rate on glucose ..	0·0	2·4	6·2	6·5	7·1	7·8	8·5	4·0	4·0	3·5	3·3	2·7	2·2	2·0	1·8	1·5

TABLE III

Showing daily dry weight (in mgm.) and growth rate of Phyllosticta cycadina, P. artocarpina *and* Pestalotia mangiferæ *on lactose as well as a mixture of its hydrolytic products*

	DAYS															
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th	13th	14th	15th	16th
Phyllosticta cycadina—																
1 Daily dry wt. on lactose ..	0·0	2·3	7·5	13·0	20·0	25·0	29·3	32·0	34·0	35·6	36·5	37·2	37·7	38·0	38·2	28·2
2 Daily dry wt. on mixture of glucose and galactose	0·0	4·5	13·0	22·0	33·0	47·5	56·0	64·0	70·0	73·5	76·6	79·0	81·0	82·3	83·0	83·5
3 Growth rate on lactose ..	0·0	2·3	5·2	5·5	7·0	5·0	4·3	2·7	2·0	1·6	0·9	0·7	0·5	0·3	0·2	..
4 Growth rate on mixture of glucose and galactose	0·0	4·5	8·5	9·0	11·0	14·5	8·5	8·0	6·0	3·5	3·1	2·4	2·0	1·3	0·7	0·5
Phyllosticta artocarpina—																
1 Daily dry wt. on lactose ..	0·0	3·5	7·5	12·0	17·4	23·5	30·5	36·2	40·2	43·3	46·0	48·0	49·2	49·9	50·4	50·5
2 Daily dry wt. on mixture of glucose and galactose	0·0	4·0	14·0	26·0	40·0	52·0	61·0	68·5	76·0	83·1	89·5	92·2	94·5	96·5	98·0	98·5
3 Growth rate on lactose ..	0·0	3·5	4·0	4·5	5·4	6·1	7·0	5·7	4·0	3·1	2·7	2·0	1·2	0·7	0·5	0·1
4 Growth rate on mixture of glucose and galactose	0·0	4·0	10·0	12·0	14·0	12·0	9·0	7·5	7·5	7·1	6·4	2·7	2·3	2·0	1·5	0·5
Pestalotia mangiferæ—																
1 Daily dry wt. on lactose ..	0·0	2·9	6·8	11·0	14·5	18·0	21·2	24·0	26·0	27·6	28·7	29·6	30·4	30·9	31·2	31·2
2 Daily dry wt. on mixture of glucose and galactose	0·0	3·5	9·5	16·5	24·0	29·0	33·8	38·3	41·3	44·1	46·3	48·3	50·1	51·6	52·8	53·5
3 Growth rate on lactose ..	0·0	2·9	3·9	4·2	3·5	3·5	3·2	2·8	2·0	1·6	1·1	0·9	0·8	0·5	0·3	..
4 Growth rate on mixture of glucose and galactose	0·0	3·5	6·0	7·0	7·5	5·0	4·8	4·5	3·0	2·8	2·2	2·0	1·8	1·5	1·2	0·7

organisms and glucose or galactose were, therefore, not formed in the culture medium. The above results also established that lactose was finished on 9th and 12th day by *Phyllosticta cycadina* and *P. artocarpina* respectively, while *Pestalotia mangiferæ* could not utilize all the lactose of the medium even in 15 days. The daily dry weight and growth rate of these organisms on lactose and a mixture of its hydrolytic products is recorded in Table III.

It is evident from Table III that the growth rate of *P. cycadina* and *P. artocarpina* increased upto 5th and 7th day respectively, while the rate of growth of *Pestalotia mangiferæ* was never very high and was not significantly different between 2nd and the 8th day. These fungi showed much better growth on a mixture of glucose and galactose (hydrolytic products of lactose) than on lactose. Chromatographic analysis of the medium containing this mixture revealed that all the three fungi assimilated glucose (R_f 0·67) one day earlier than galactose (R_f 0·65). Glucose was assimilated in 9, 11 and 13 days by *P. cycadina*, *P. artocarpina* and *P. mangiferæ* respectively.

Discussion

Present investigations clearly showed that sucrose and maltose were good carbon sources for all the three organisms. Similar results with these two sugars were also obtained by Herrick (1940), Blank and Talley (1941), Cantino (1949), Fergus (1952), Wolf (1953) and Agarwal (1955) for the fungi investigated by them. Daily chromatographic analysis of the medium indicated that all the present fungi assimilated sucrose and maltose through a hydrolytic pathway. The results further showed that out of the two hydrolytic products of sucrose, glucose was assimilated three days earlier than fructose by the two species of *Phyllosticta*, though both glucose and fructose were present in their hosts. Separate investigations by Bilgrami (1956) also confirmed these results and it was observed that both the species of *Phyllosticta* gave better mycelial growth on glucose than on fructose.

Pestalotia mangiferæ differed from the two species of *Phyllosticta*, because it was a slow hydrolysing fungus, and it consumed both the hydrolytic products of sucrose simultaneously. Hawker (1947) reported that the amount of mycelium produced by *Melanospora destruens* was different when this fungus was grown on sucrose or on a mixture of glucose and fructose. Her results indicated that it was not always essential that a complex carbohydrate (*e.g.*, sucrose) or its hydrolytic products (*e.g.*, mixture of glucose and fructose) should always be equally good sources. The results obtained with the present organisms differed from those of Hawker (1947). It was observed that there was no significant difference in dry weight of these fungi on sucrose or on a mixture of its hydrolytic products. Records on daily rate of assimila-

tion of sucrose had indicated that *Pestalotia mangiferæ* showed slightly delayed response on sucrose than on a mixture of glucose and fructose. It appears that this may be due to delayed and slow activity of the enzyme invertase. Chromatographic analysis of the medium on which *P. mangiferæ* was growing also confirmed that glucose and fructose were not formed in the culture medium till 3rd day and growth appeared after that period. Thus it is also clear that sucrose is not assimilated by these organisms till it is hydrolysed. *Pestalotia mangiferæ* showed delayed response on maltose also, as the growth of this fungus started on the 4th day, though glucose (hydrolytic product of maltose) was detected in the medium on the 6th day. It appears that whatever glucose was formed on the 4th and 5th day was entirely taken up by this organism, so that no trace was left in the medium and hence it could not be detected chromatographically. The behaviour of lactose was different from that of sucrose or maltose as it was not only a poor source of carbon, but was assimilated through a non-hydrolytic pathway and in this respect the present results seem to confirm the observations of Margolin (1942).

The present results also established that the different disaccharides may differ in their behaviour, because it was clearly observed that the growth on sucrose was similar, on maltose it was better and on lactose it was poorer than on their hydrolytic products.

Chromatographic studies showed the presence of maltotriose (an oligosaccharide) which appears to have been synthesized during the process of assimilation and which seems to play an important role in making maltose a better carbon source than its hydrolytic product. It may be mentioned that recently various workers, including Giri, Nigam and Srinivasan (1954) as well as Buston and Jabbar (1954), have reported that various fungi could play an important part in enzymic synthesis of oligosaccharides from disaccharides. The exact role of maltotriose in assimilation of maltose is, however, not clearly understood by any investigator. The present investigations indicate that these fungi were capable of synthesizing oligosaccharide only from maltose and not from the other two disaccharides, *viz.*, sucrose and lactose.

SUMMARY

Phyllosticta cycadina, P. artocarpina and *Pestalotia mangiferæ* were isolated from the leaf spot diseases of *Cycas revoluta, Artocarpus heterophyllus* and *Mangifera indica* respectively. A detailed study of the assimilation of sucrose, maltose and lactose by these organisms was undertaken. Sucrose and maltose were assimilated through a hydrolytic pathway. *Pestalotia mangiferæ* utilized both glucose and fructose simultaneously, but the two

species of *Phyllosticta* behaved differently and consumed glucose three days before the other hydrolytic product of sucrose (*viz.*, fructose). Synthesis of an oligosaccharide (maltotriose) was recorded during the assimilation of maltose. Lactose was used through a non-hydrolytic pathway and was found to be a poor source but a mixture of its hydrolytic products supported good growth.

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