PHYSIOLOGICAL STUDIES ON CERTAIN FUNGI CAUSING LEAF-SPOT DISEASES

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Introduction

Leaves are the chief centres of activity which discharge various physiological functions in the chlorophyllous plants. These plant structures have received much attention of plant morphologists and physiologists, but the plant pathologists have not devoted full attention towards them. A keen and constant observation of any apparently healthy plant reveals that at some stage or the other, some of its leaves exhibit distinct leaf spots. The magnitude of this expression depends upon several factors including the nature of the host, invading capacity of the pathogens, environmental conditions and the age of the leaves, etc. The total exposed area of the entire foliage system is enormously large enough to trap the fungal spores. Bald (1952) observed that stomatal droplets induce the germ tube of the leaf invading fungi to pass into the stomata and infect the leaf.

Recent results of many other investigators also amply indicate that several nutritive substances ooze out over the leaf surface, promote spore germination and facilitate the entry of germ tube into the host tissue. These findings indicate that in many cases host ingredients are responsible for determining the initiation of infection. Further propagation of disease depends upon the capacity of the pathogen to derive its food from the host tissue. This paper deals only with the role of carbohydrates on the growth of some pathogenic fungi causing leaf spot diseases. Carbohydrates, being the chief metabolic products of the photosynthetic plants, have special importance in the nutrition of disease causing organisms. Inside the host they exist in two states: (i) simple easily utilizable forms; (ii) complex forms.

The food of the first category is assimilated as such and the pathogens have not to strain on that account while the utilization of complex substances is accomplished by those micro-organisms only which can satisfactorily break them.

The utilization of some common carbohydrates by a number of pathogenic fungi representing diverse groups of Deuteromycetes has been studied.

Materials and Methods

The following fungi were isolated from the leaves of their respective hosts:

1. *Pestalotia banksiana* from *Grevillea robusta*
2. *Pestalotia citri* from *Citrus maxima*
3. *Phyllosticta artocarpina* from *Artocarpus heterophyllus*
4. *Phyllosticta mortoni* from *Mangifera indica*
5. *Phyllosticta morifolia* from *Morus alba*
6. *Phyllosticta carica-papayae* from *Carica papaya*
7. *Alternaria tenuis* from *Lycopersicon esculentum*
8. *Colletotrichum capsici* from *Scindapus pictus*
9. *Colletotrichum gloeosporioides* from *Punica granatum*
10. *Pestalotiopsis glandicola* from *Thea sinensis*
11. *Cercosporina ricinella* from *Ricinus communis*
12. *Curvularia penniseti* from *Pennisetum typhoideum*

A medium containing 10·0 g glucose, 3·5 g KNO_3, 1·75 g KH_2PO_4, 0·75 g $MgSO_4.7\,H_2O$, 100 μg thiamine, 10 μg biotin dissolved in one litre of double distilled water was employed for physiological studies. All the experimental glassware was of Pyrex-make. Extra pure chemicals supplied by B.D.H., E. Merck or Difco were employed. The substrate consisted of autoclaved or steam-sterilized liquid nutrient. Superficially cut agar discs from 6–10 days old colonies were used as inocula. The inoculated flasks were incubated at 25° C. At the end of each incubation period the fungal colonies were thoroughly washed and were subsequently filtered on previously dried and weighed filter-paper No. 42. Fungus containing filter-papers were subjected to 65° C. temperature (in electric ovens) for two days after which they were cooled and accurately weighed. Dry weight results were used for comparative purposes. Each set was run in triplicate. Average of three dry weights was taken as standard value for comparison of growth. The efficiency of growth has been grouped in three broad categories, *viz.*, good, moderate and poor. The medium was chromatographically analyzed in order to study the daily changes in the sugar contents of the medium. The technique of Giri *et al.* (1953) was employed for this purpose. *n*-Butanol-acetic acid-water (4 : 1 : 5) or *n*-butanol-pyridine-water (4 : 6 : 3) were used as solvents. The chromatograms with single central wick were run for about 7 hours. They were subsequently dried at room temperature for three hours and were then sprayed with a mixture of aniline-diphenylamine phosphoric acid. The sprayed chromatograms were again dried in air for about 45 minutes and were finally

suspended in electric oven at 110° C. for 60–90 seconds. After appearance of bands the Rf values of different sugars were calculated. Occasionally the solutions of known sugars were also placed on the test chromatograms to facilitate the identification of various sugars.

In order to determine the nature of synthetic oligosaccharides, the approximate area containing the new oligosaccharide was separated and eluted in 5·0 c.c. of hot water. The eluted solution was divided into two equal parts. One fraction was partially hydrolysed while the other part was totally hydrolysed with conc. HCl. The hydrolysed solutions were again assayed chromatographically.

Results

The three grades of growth (*viz.*, good, moderate and poor) of different fungi attained on various carbohydrates is recorded in Table I.

A review of Table I shows that starch, sucrose, maltose, glucose and fructose were invariably good sources for all the pathogens while their response on cellulose and melibiose was poor. The efficiency of raffinose, lactose, galactose and mannose ranged from good to poor. The results also express that different species of the same genus could behave alike or differently towards a particular source of carbon.

Chromatographic Observations

The chromatographic analysis of the starch-medium revealed that in all cases except in *Colletotrichum capsici* maltose and glucose were distinctly traced in the culture solution. An oligosaccharide (Rf 0·42) was also produced in the culture solution. *Phyllosticta artocarpina* and *P. mortoni* synthesized two additional oligosaccharides. Neither the hydrolytic products of cellulose (*viz.*, cellobiose and glucose) nor any synthetic oligosaccharides were spotted during the utilization of this substance. Trisaccharide, raffinose was gradually transformed into a mixture of melibiose and fructose by all the organisms except *Colletotrichum capsici* which used it at a very slow rate. Invariably all the fungi converted sucrose into a mixture of glucose and fructose and in every case the former component was consumed earlier. This evidently shows their comparative preference for glucose. At least one oligosaccharide was synthesised during the growth of these fungi on sucrose solution. Most of the species of *Phyllosticta* were also capable of forming an additional oligosaccharide (Rf 0·30).

Maltose medium showed distinct presence of glucose. The rate of its conversion was generally rapid by all the isolates but the corresponding rate of consumption of glucose varied with different isolates. One or two transient oligosaccharides were also produced in the medium. The other glucose-yielding disaccharide, *viz.*, cellobiose, was disintegrated at a very slow rate and the hydrolytic product (*viz.*, glucose) was detected only occasionally in very feeble quantity. Synthetic oligosaccharides were not spotted in any of the culture flasks. None of the

TABLE I

Representing the growth of twelve leaf spot causing fungi on various carbon sources

	Pestalotia banksiana	*Pestalotia citri*	*Phyllosticta artocarpina*	*Phyllosticta mortoni*	*Phyllosticta morifolia*	*Phllosticta caricapapayae*	*Alternaria tenuis*	*Colletotrichum capsici*	*Colletotrichum glotosporioides*	*Pestalotiopsis glandicola*	*Cercosporina ricinella*	*Curvularia penniseti*
Starch ..	G	G	G	G	G	G	G	M	G	G	G	G
Cellulose ..	P	P	P	P	P	P	..	..	..	..	..	..
Raffinose ..	G	G	M	M	G	G	M	P	G	G	..	..
Sucrose ..	G	G	G	G	G	G	G	G	G	G	G	G
Maltose ..	G	G	G	G	G	G	G	G	G	G	G	G
Cellobiose ..	P	M	P	P	P	P	..	..	..	..	..	..
Lactose ..	P	P	M	P	P	P	G	G	M	P	P	P
Melibiose ..	P	P	P	P	P	P	..	..	..	..	..	..
Glucose ..	G	G	G	G	G	G	G	G	G	G	G	G
Fructose ..	G	G	G	G	G	G	G	G	G	G	G	G
Galactose ..	G	M	M	G	M	G	G	G	P	M	G	G
Mannose ..	M	G	P	G	M	M	G	P	G	P	M	P

G has been used for 'good' ; M for 'moderate' ; P for 'poor'.

organisms under study were able to consume the entire cellobiose within the incubation time and a significant portion was left unfinished in the medium even at the end of the incubation period. The hydrolytic products of melibiose and lactose (*i.e.*, glucose and galactose) were spotted infrequently on lactose medium while they were not produced on melibiose medium. In all the cases a significant fraction of melibiose was left unconsumed in the medium. No trace of any synthetic oligosaccharides was recorded on either of the above media.

Amongst the hexose sugars the rate of utilization of glucose and fructose was quite rapid by all the pathogens. Glucose was finished within 4–6 days while fructose was exhausted by the 8th day. The rate of consumption of galactose and mannose varied with the organism and in some cases they were left unfinished even at the end of the incubation period. The comparative rate of utilization in every case corresponded with the hyphal output.

A correlative study of dry weight and chromatographic results showed that the rate of utilization of oligo- and polysaccharides was dependent on their rate of disintegration. Those complex carbohydrates which were hydrolysed quicker (*e.g.*, starch, sucrose or maltose) were always more favourite sources. Distinct formation of oligosaccharides was also a consistent feature in the above cases.

Discussion

Studies dealing with leaf-spot diseases are very spotty and, therefore, no co-ordinated account dealing with physiological aspects is available. The plant pathogens mostly come in contact with complex type of food in the host tissues. The survival and further propagation of the disease depends upon the success of the growing mycelium in absorbing the nutrients. An analysis of the hosts showed that at least four carbohydrates, *viz.*, starch, sucrose, glucose and fructose, were present in all of them. The results confirm that invariably they were satisfactory sources for the present pathogens, which were, therefore, well adapted to utilize those carbohydrates which existed in the hosts. Maltose was a good source even though it was lacking in the healthy tissues. Better response on maltose can be attributed to the capacity of these pathogens to use starch satisfactorily because maltose is produced during its breakdown. Most of the present organisms attained comparatively better growth on starch and maltose than on its basic structural constituent, *i.e.*, glucose. Similar preferentiality was exhibited by several other fungi including *Saprolegnia delica* (Bhargava, 1945), *Macrochytrium botrydioides* (Crasemann, 1954), *Fusarium oxysporum f. nicotianae* (Wolf, 1955), and species of *Phyllosticta* (Bilgrami, 1962). The fungi included here failed to adapt themselves to cellulose and this evidently shows that *cellulase* activity was limiting in the pathogenic microorganisms. With the exception of *Colletotrichum capsici* all other fungi were capable of disintegrating raffinose satisfactorily. The formation of only melibiose and fructose during the utilization of

this trisaccharide shows efficient β-fructosidase activity. Rapid utilization of fructose and accumulation of melibiose has also been reported for *Ceratocystis fimbriata* and *Thielaviopsis basicola* (Wilson and Lilly, 1958). It was interesting that with the exception of melibiose all other components of raffinose were individually good sources.

Wilson and Lilly (1958) as well as Wilson (1960) have also listed several fungi which are able to synthesize transient oligosaccharides during the utilization of maltose. This distinctly shows that cleavage of maltose is accomplished by transglycosidases. Pan *et al.* (1953) and Giri *et al.* (1953) have shown that along with hydrolysis, transglycosidases also cause replacement reaction of glycosidic bond due to which there may be synthesis of certain oligosaccharides as intermediates during the utilization of oligo- or polysaccharides. Conversion of starch and maltose media to a mixture of carbon sources might be one of the reasons for the superiority of these substances over glucose. Instances are, however, not lacking where certain fungi are completely incapable to thrive on maltose, *e.g.*, *Polychytrium aggregatum* (Ajello, 1948) and *Chytridium* spp. (Crasemann, 1954). The fungi included in the present study expressed only a mild liking for cellobiose but a number of imperfect fungi including *Chalara quercina* (Beckman *et al.*, 1953), *Memnoniella echinata* (Perlman, 1948 *a*), *Colletotrichum phomoides* (Lilly and Barnett, 1953) attained better vegetative growth on cellobiose than on glucose.

It has been found that the organisms which are adapted to saprophytic mode of living, *e.g.*, some members of Chytridiales (Crasemann, 1954), Saprolegniales (Bhargava, 1945 ; Ram Dayal, 1961) and Mucorales (Raizada, 1957) failed to utilize sucrose while most of the pathogenic fungi were fully capable to assimilate it. Sucrose has been reported superior even to glucose for a number of fungi studied by Perlman (1948 *b*) as well as Misra and Mahmood (1960). Due to transglycosidation, the synthetic oligosaccharides are formed on sucrose medium also. On the basis of the analysis of these synthetic oligosaccharides Bilgrami (1962) suggested unequal availability of its component sugars (*i.e.*, glucose and fructose).

Like most of the present organisms a number of other imperfect fungi including *Sphaeropsis malorum*, *Fusarium niveum*, *Melanconium fuligineum* (Lilly and Barnett, 1953) and *Fusarium oxysporum* f. *nicotianae* (Wolf, 1955) are also known to use lactose incompletely. Limiting melibiase activity by leaf-spot pathogens appears to be responsible for poor availability of melibiose to them.

The formation of oligosaccharides on media of various carbohydrates is evidently carried by enzymes which catalyse the rupture of glycosidic linkage, uniting the various monosaccharide components. This is accomplished by introduction of a molecule of water which splits the glycosidic bond. The dissociated monosaccharide again gets attached to the original oligosaccharide and in this manner the oligosaccharide of varying chain lengths may be produced in the culture-medium.

SUMMARY

The utilization of carbohydrates by 12 pathogenic imperfect fungi was studied. The carbohydrates or their derivatives which existed in the host tissues were generally suitable sources. Synthesis of oligosaccharides on solutions of oligo- or polysaccharides was also quite common. There was no consistency in response on those substances which were lacking in the host. Factors responsible for the formation of synthetic oligosaccharides have also been discussed.

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