SECTION H, MICROBIOLOGY

A Preliminary Investigation on Carbohydrate Nutrition of the Pecan Scab Fungus, Fusicladium Effusum Wint.

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Pecan scab, incited by a fungus, *Fusicladium effusum* Wint., is a limiting factor in the production of pecan nuts throughout the pecan belt. To fully understand the disease, basic information on the nutrition of the pathogen is needed. This paper reports results of a preliminary investigation on carbohydrate nutrition of *F. effusum* in a chemically-defined medium containing various individual filter-sterilized monosaccharides and oligosaccharides. Results with autoclaved polysaccharides are also presented.

Materials and Methods

An autoclaved synthetic nutrient base solution, to which was added a filter-sterilized or autoclaved carbohydrate solution or suspension, was used as the test medium. The basal nutrient solution contained 1 g $MgSO_4 \cdot 7H_2O$, 2 g KH_2PO_4, 2 g asparagine, 1 ml thiamine soln (100μg), 1 ml biotin soln (5 μg) and 2 ml trace elements soln (0.2 mg Fe^{+++}, 0.2 mg Zn^{++}, 0.1 mg Mn^{++}) (1951, p 421) in sufficient distilled water to bring the volume to 900 ml. Forty-five ml aliquots of each 900 ml quantity were placed in each set of twenty 250 ml Erlenmeyer flasks. The flasks were plugged with foam-plastic closures and autoclaved for 20 minutes at 15 psi. After cooling, 5 ml of a solution or a suspension of a particular carbohydrate (20 g/100 ml), previously sterilized by passage through a fritted-glass filter or autoclaving, was aseptically added to each of 20 flasks per carbohydrate. Sterilization by filtration was used in the case of the water soluble monosaccharides and oligosaccharides. The water insoluble polysaccharides were autoclaved separately from the basal solution to prevent carbohydrate reactions which form toxic or growth-promoting compounds (Cochrane, 1958). Separate autoclaving does not prevent partial hydrolysis, however, and this introduces an error which must be taken into account when results are interpreted. Sterilization by filtration prevents hydrolysis and undesired reactions between carbohydrates and other constituents of the medium, especially phosphates and amino acids (Cochrane, 1958). Glass filtration also prevents pH changes which occur when Seitz filters are used (Browne, 1942).

The initial pH of the medium from 1 flask in each set of 20 per carbohydrate was electrometrically determined. Individual 7 mm mycelial discs, cut from peripheries of 4-6 wk-old *F. effusum* colonies on peptone-dextrose agar, were placed in each flask. The flasks were incubated for 21 days at

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75F ±¹, an optimum temperature for growth of this fungus (Nolen, 1926). The mycelium from 10 representative flasks in each set of 20 per carbohydrate was harvested onto tared filter papers, washed repeatedly with distilled water, oven dried (75C) for about 18 hours, cooled, weighed and mycelial weights determined. The pH of the filtrates was determined and compared with the initial pH values of the medium.

Results

Differential growth responses were obtained (Table 1). The greatest growth occurred on 2 hexoses, fructose and mannose. Of the oligosaccharides tested, a trisaccharide, raffinose, was utilized best. A disaccharide, sucrose, followed raffinose. These 2 compounds were utilized much more poorly than were fructose and mannose. A polysaccharide, "soluble" starch (partially depolymerized), followed sucrose. The pentoses, a methyl pentose (rhamnose) and most of the polysaccharides tested were poorly utilized. Sugar alcohols were utilized only slightly better. For most of the carbohydrates tested, no great changes in pH of the medium were found.

Discussion and Conclusions

For most fungi, the hexoses d-fructose, d-mannose and d-glucose are equivalent for growth (Cochrane, 1958). The results from the currently reported investigation indicate that fructose and mannose may be actually superior to glucose for growth of *F. effusum*. A longer growth period with glucose may have shown that glucose is utilized equally but over a longer period. The poor utilizaton of galactose was expected as this compound is a poor source of carbon for most fungi (Cochrane, 1958).

Of the pentoses, fungi generally utilize d-xylose preferentially but *F. effusum* grew poorly on filter-sterilized xylose. This is probably a true reflection on actual utilizaton of xylose because xylose is known to be converted to furfural during sterilization by autoclaving. *F. effusum* also grew poorly on filtered arabinose. As is generally true with fungi, arabinose was utilized less readily by *F. effusum* than was the hexose sugar glucose.

In general, *F. effusum* utilized oligosaccharides less well than it utilized hexoses but it utilized the oligosaccharides better than the pentoses arabinose and xylose. The fungus utilized disaccharides less than the single trisaccharide tested, raffinose. Filter-sterilized maltose was utilized very poorly, if at all. Apparently *F. effusum* cannot readily hydrolyze maltose. The fungus can utilize lactose only slightly better. Lactose is a poor source of carbon for this fungus as it is for many fungi. Apparently *F. effusum* produces enzymes which attack the beta glycoside linkage in lactose but which cannot attack the alpha glycoside linkage in maltose. Though lactose is probably hydrolyzed to glucose and galactose, the fungus grows poorly because it can utilize only the glucose moeity readily. The utilization of raffinose almost approached that of mannose and fructose. Apparently enzymes which readily hydrolyze raffinose were produced. Probably sucrase is produced because *F. effusum* readily utilized sucrose. Sucrase hydrolyzes raffinose to fructose and melibose. The melibose may have been hydrolyzed in turn by other enzymes, such as melibiase, to produce glucose and galactose. Because fructose and glucose are readily utilized, growth was good on raffinose.

Despite sterilization of the poysaccharides by autoclaving, all, except "soluble" starch, were poorly utilized. The apparent slight utilization of these compounds may have been due to utilization of possible degradation

TABLE 1. GROWTH OF FUSICLADIUM EFFUSUM IN A SYNTHETIC LIQUID MEDIUM CONTAINING INDIVIDUAL CARBOHYDRATES.

Carbohydrates	Initial pH of medium	Final pH of medium	Av. wt. (g) of oven-dried mycelium/flask
Monosaccharides			
Pentoses			
d-Arabinose	4.7	4.3	0.0074
d+ Xylose	4.4	4.5	0.0076
Methyl pentoses			
l+ Rhamnose	4.6	3.8	0.0059
Hexoses			
d-Fructose	4.5	5.0	0.1648
d+ Mannose	4.5	4.5	0.1521
d+ Glucose	4.4	4.5	0.0260
d+ Galactose	4.7	4.5	0.0078
Oligosaccharides			
Disaccharides			
d+ Sucrose	4.4	4.7	0.0611
d+ Cellobiose	4.4	4.8	0.0315
d+ Lactose	4.5	4.3	0.0129
d+ Maltose	4.4	4.5	0.0022
Trisaccharides			
d+ Raffinose	4.4	4.2	0.1160
Polysaccharides			
Starch ("soluble")	4.7	4.6	0.0442
Amylopectin	4.4	5.4	0.0146
Pectin	4.6	3.8	0.0119
Gum Arabic	4.2	4.7	0.0056
Inulin	4.7	4.6	0.0046
Sugar Alcohols			
d-Sorbitol	4.4	5.2	0.0459
d-Mannitol	4.7	5.4	0.0403
Inositol	5.0	4.6	0.0261
Glycerol	4.6	4.5	0.0204
Dulcitol	4.8	4.7	0.0048
None (Control)	4.7	4.9	0.0014

products formed during autoclaving or to the presence of contaminating heat-stable growth factors. Starch is generally a good source of carbon for fungi and is often a better source than the readily utilized hexose sugar glucose. This was true for *F. effusum* also. This fungus utilized "soluble" starch better than glucose but not as well as fructose, mannose, raffinose or sucrose. Because the fungus utilizes maltose very poorly one would expect it to utilize starch very poorly also. The fair utilization of starch suggests that it may have been degraded to glucose by heat and that trace amounts of growth factors were prseent also. Whether this pathogen can utilize native starch present in host tissues remains to be determined. Because of the excellent utilization of fructose and the presence of beta glycoside linkages between the fructofuranose units of inulin one might expect that inulin would be readily utilized. The very poor utilization of inulin implies that this fungus does not produce the beta glycosidases required to hydrolyze inulin.

The pH of culture media usually changes during culture of microorganisms. A drop in pH indicates the formation of organic acids or absorption of cations while an increase in pH indicates formation of am-

monia or absorption of anions. The stability of pH in mosts cases in this investigation implies that these factors are not of great importance when culturing *F. effusum*.

Because an arbitrary incubation period was used in this investigation, a source of error in obtaining information on intrinsic utilizability of compounds was introduced. A compound not readily utilized may be utilized adaptively after a long lag period. The determination of a compound as utilizable or non-utilizable, therefore, may depend on the incubation period allowed. Multiple harvest dates should be planned in this type of work.

The use of an organic nitrogen source, asparagine, introduced an additional source of carbon but this apparently had little effect on the results because of the minimal growth in the carbohydrate-free control flasks. An inorganic nitrogen source will be used in future studies on carbohydrate nutrition.

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