SECTION H, MICROBIOLOGY

Utilization of Carbohydrate Compounds by the Pecan Scab Fungus, Fusicladium Effusum Wint[1].

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Fusicladium effusum Wint. is the casual agent of pecan scab, one of the most serious diseases of the pecan throughout most of the growing range of the tree. To fully understand the disease, basic information is needed on all aspects of the nutrition of the pathogen. A preliminary paper on utilization of carbohydrates by *F. effusum* has appeared (Barnes and Adams, 1964). This paper reports on additional research on carbohydrate nutrition of the fungus. Results with fritted glass-sterilized monosaccharides, oligosaccharides, and some polysaccharides are presented. Results with some autoclave-sterilized polysaccharides are also reported.

MATERIALS AND METHODS

The test medium was an autoclaved, chemically defined nutrient solution, to which was added a glass filter-sterilized, water-soluble carbohydrate or an autoclaved water-insoluble carbohydrate. The medium contained 1 g $MgSO_4 7H_2O$, 3 g KNO_3, 2g KH_2PO_4, 100 μg thiamine, 0.2 mg Fe^{+++}, 0.2 mg Zn^{++} and 0.1 mg Mn^{++} in sufficient distilled water to bring the volume to 800 ml. Forty-ml aliquots of each 800-ml quantity were dispensed into sets of twenty 250-ml Erlenmeyer flasks. The flasks of basal nutrient solution were plugged with foam-plastic closures and autoclaved at 121 C for 20 min. After cooling, 5 ml of a sterile solution or suspension of an individual carbohydrate, at a concentration of 10 g/50 ml, was aseptically pipetted into each of 10 flasks of sterile solution. Sterilization by glass filtration was employed in the case of all of the water-soluble compounds. The water-insoluble compounds were autoclaved separately from the basal medium to prevent reactions between constituents of the medium which may form compounds which are toxic to or promote the growth of fungi (Cochrane, 1958). Autoclaving of water-insoluble carbohydrates separate from the medium does not prevent partial hydrolysis and this introduces a factor which must be considered when results are interpreted. Sterilization by filtration prevents hydrolysis. Glass filtration also prevents pH changes which can occur when Seitz filters are used (Browne, 1942).

Most of the carbohydrates tested were obtained from the Nutritional Biochemicals Corporation. Sorbitol and amylose were obtained from the California Corporation for Biochemical Research. To determine a good test pH, test batches of the basal medium were adjusted to various pH values ranging from 3.0 to 6.5. The fungus grew best in the range 3.5 to 4.7 (Table I). Because of these results, and because the fungus had grown well in the same medium at pH 4.5 in earlier tests (Barnes and Adams, 1964), pH 4.5 was selected as the test pH. The addition of a carbohydrate changed the pH only slightly, if at all. One flask from each set of 10 per test compound was used for a determination of the initial pH with a Beckman Zeromatic II pH Meter.

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[1]This research was supported by NIH funds (PH-EF-308 and UI-277-03) and by Oklahoma Agricultural Experiment Station Project S-1284. Portion of a Master's thesis by the senior author.

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TABLE I. GROWTH OF *Fusicladium effusum* AT DIFFERENT pH VALUES WITH CERTAIN CARBOHYDRATES IN A SYNTHETIC MEDIUM.

Carbohydrate	Initial pH of Medium	Final pH of Culture Filtrate	Av. Wt. (g) of Oven-Dried Mycelium/Flask
Monosaccharide			
D(—)Fructose	3.0	3.1	0.0126
	3.5	5.6	0.0526
	4.0	5.7	0.0480
	4.7	6.1	0.0677
	5.0	5.2	0.0195
	5.5	5.5	0.0105
	6.0	6.0	0.0102
	6.5	6.4	0.0087
Oliogosaccharide			
D(+)Sucrose	3.0	5.1	0.0072
	3.5	6.6	0.1400
	4.0	6.6	0.1725
	4.5	6.3	0.0872
	5.0	5.5	0.0214
	5.5	5.6	0.0177
	6.0	6.0	0.0125
	6.5	6.4	0.0087
Polysaccharide			
Dextrin	3.0	5.8	0.0525
	3.5	6.3	0.0739
	4.0	6.3	0.0634
	4.5	6.3	0.0618
	5.0	6.2	0.0504
	5.5	6.1	0.0391
	6.0	6.3	0.0273
	6.5	6.5	0.0222

The inoculum used in these tests was a mycelial suspension prepared from a 4- to 5-week-old culture of *F. effusum* on peptone-dextrose agar. The use of a mycelial suspension rather than a single mycelial disk as used in earlier studies (Barnes and Adams, 1964) prevented wide variations in yields between replications. A spore suspension could not be used as this fungus has not been induced to sporulate well in culture.

Ten 7-mm disks were blended in 50 ml of sterile distilled water in a sterile Waring Blendor microcontainer for 45 seconds and 5 ml of the suspension was added to each flask. All tests were incubated for 28 days at 25 C, an optimum temperature for growth of this fungus (Nolen, 1926). Mycelium was harvested by filtration onto filter papers previously dried in an oven at 80 C and weighed. Harvested mycelium and filter papers were uniformly and repeatedly washed with distilled water and then oven dried for 24 hr at 80 C. Before final weighings, each harvest was allowed to cool in a desiccator. The filtrates from each test carbohydrate were pooled and the pH was determined with a pH meter.

RESULTS

In the tests to determine a suitable pH level for growth of *F. effusum* it was found that, regardless of the carbohydrate source, the fungus grew

better at quite low pH values than at values approaching neutrality (Table I). When D(—)fructose was used in the medium, the greatest growth occurred when the initial pH was 4.7. In the case of D(+)sucrose, the greatest growth occurred when the initial pH was 4.0. Very good yields of mycelium were obtained with D(+)sucrose between pH 3.5 and 4.5. With dextrin, the greatest yield was obtained at a pH of 3.5. Good yields were obtained between pH 3.5 and pH 5.0. With sucrose and fructose, the lowest yields were obtained when the initial pH was 3.0 or 6.5. With all three carbohydrates the lowest yields were obtained at pH 6.5.

Differential growth responses were obtained. The greatest growth occurred on the hexose sugar, mannose (Table II). Other hexoses which were good carbon sources were D(—)fructose and D(+)glucose. Of the oligosaccharides tested, D(+)raffinose, a trisaccharide, was utilized best and was the second best utilized compound. A disaccharide, D(+)sucrose, closely followed raffinose. The pentoses, two methyl pentoses, sugar alcohols, and some of the polysaccharides were poorly utilized. Of the pentoses, D(—)ribose provided the greatest growth. Of the sugar alcohols, D-mannitol and D-sorbitol provided the greatest growth.

The pH of culture filtrates changed little in the case of poorly utilized compounds but it approached neutrality in the case of compounds which were well utilized.

Discussion and Conclusions

In this and earlier work (Barnes and Adams, 1964), yields of *F. effusum* were greatest when the initial pH of the synthetic medium was between 3.5 and 4.7. Most plant pathogens grow best in media with an initial pH of 5.0 to 6.5 (Cochrane, 1958). *F. effusum* is an exception as it grows best at a pH below 5.0 and grows very poorly at pH 6.5. A closely related fungus, *F. dendriticum*, has been reported to grow best at pH 5.8 (Fothergill and Ashcroft, 1955). Other investigators reported that *F. dendriticum* grew best between pH 5.1 and 6.4 (Leben and Keitt, 1948).

Like most fungi, *F. effusum* utilized hexoses to a greater extent than any other type of carbohydrate. D(+)mannose was previously reported to be an excellent source of carbon for this fungus (Barnes and Adams, 1964) and this was confirmed in the presently reported work. For the great majority of fungi, D(+)mannose and D(—)fructose are equivalent to D(+) glucose for growth but D(+)mannose is superior to both for growth of *F. effusum*. The poor utilization of D(+)galactose and L(—)sorbose was expected as these compounds are generally poorly utilized by fungi (Cochrane, 1958).

Of the pentoses, fungi generally utilize D(+)xylose best but *F. effusum* did not utilize filter-sterilized D(+)xylose. Xylose is converted to furfural, a fungal toxicant, during autoclaving and poor utilization on filter-sterilized material would indicate a true picture of poor utilization (Cochrane, 1958). D(—)ribose was the only pentose utilized by *F. effusum*. A methyl pentose, L(+)rhamnose, was also utilized. Relatively few reliable studies have been made with either compound. It is not known whether many fungi utilize these compounds or not.

The oligosaccharides as a class are intermediate in utilization by *F. effusum*. Lactose, the least fungus-utilized disaccharide, is composed of one glucose unit and one galactose unit. D(+)lactose is poorly utilized by *F. effusum*. This indicates that lactase may be produced but only in a minute quantity. Utilization of maltose implies production of maltase. The good utilization of D(+)sucrose would indicate sucrase production by the fungus. Further support of this hypothesis is evidenced by the excellent utilization of D(+)raffinose (fructose-glucose-galactose). Su-

TABLE II. GROWTH OF *Fusicladium effusum* IN A CHEMICALLY DEFINED MEDIUM CONTAINING SINGLE CARBOHYDRATES

Carbohydrate Compounds	Initial pH of Medium	Final pH of Culture Filtrate	Av. Wt. (g) of Oven-Dried Mycelium/Flask
Monosaccharides			
Pentoses			
D(—)Lyxose	4.5	4.8	0.0009
D(+)Xylose	4.5	5.1	0.0010
D(—)Arabinose	4.5	4.7	0.0027
L(+)Arabinose	4.5	4.6	0.0010
D(—)Ribose	4.5	5.0	0.0441
Methyl Pentoses			
L(—)Fucose	4.7	4.9	0.0014
L(+)Rhamnose	4.5	4.9	0.0843
Hexoses			
L(—)Sorbose	4.7	—	0.0034
D(+)Galactose	4.5	—	0.0565
D(+)Glucose	4.6	—	0.0691
D(—)Fructose	4.6	—	0.1331
D(+)Mannose	4.5	—	0.2682
Heptoses			
D(—)Sedoheptulose	4.7	4.9	0.0074
Oligosaccharides			
Disaccharides			
D(+)Cellobiose	4.5	6.0	0.0044
D(+)Lactose	4.5	4.6	0.0486
D(+)Turanose	4.5	6.2	0.0538
D(+)Melibiose	4.3	6.4	0.1197
D(+)Maltose	4.7	6.5	0.1240
D(+)Trehalose	4.5	6.4	0.1719
D(+)Sucrose	4.6	6.7	0.1792
Trisaccharides			
D(—)Melezitose	5.0	6.7	0.1523
D(+)Raffinose	4.6	6.7	0.1795
Sugar Alcohols			
i-Erythritol	4.5	4.9	0.0048
Adonitol	4.5	4.5	0.0211
Glycerol	4.5	4.7	0.0351
D-Dulcitol	4.5	5.8	0.0430
i-Inositol	4.5	4.8	0.0480
D-Mannitol	4.6	6.4	0.0542
D-Sorbitol	4.5	6.0	0.0594

TABLE II, continued

Polysaccharides*			
Glycogen	4.5	5.0	0.0004
Pectin	4.5	3.6	0.0010
Gum Arabic	4.5	4.9	0.0082
Amylopectin	4.5	5.5	0.109
Gum Tragacanth	4.5	5.6	0.0146
Xylan	4.5	6.1	0.0208
Starch ("Soluble")	4.5	6.3	0.0317
Amylose	4.5	—	0.0403 **
Dextrin	4.7	5.7	0.0781
Inulin	4.5	6.8	0.1728
None (Control)	4.5	5.0	0.0040

* All polysaccharide compounds, except inulin, were autoclaved separate from the basal medium. Inulin was dissolved in hot water and filter-sterilized.

** Each flask in this set was "inoculated" with one 7-mm disk of *F. effusum* cut from a colony growing on an agar medium.

crase would split off fructose from the molecule leaving melibiose, a compound readily utilized by *F. effusum*. This suggests formation of melibiase by the fungus. The utilization of trehalose indicates production of trehalase. The incomplete utilization of melezitose suggests the lack of an enzyme which would hydrolyze turanose but the details of the reaction are lacking (Cochrane, 1958). Turanose is utilized somewhat so production of a turanose-hydrolyzing enzyme is indicated.

Because of possible heat degradation during autoclaving, results with autoclaved polysaccharides must be interpreted with care. The slight utilization of most of the polysaccharides may indicate some heat degradation into utilizable units or production of hydrolyzing enzymes. Utilization of filter-sterilized inulin implies production of inulase. Whether this pathogen can utilize native starch and other complex carbohydrates in the pecan host, under natural conditions, remains to be determined.

The pH of culture media usually changes during culture of microorganisms. A drop of pH indicates the formation of organic acids or absorption of cations while an increase of pH indicates formation of ammonia or absorption of anions. The rise of pH when compounds were utilized in this investigation would probably relate to the latter.

Additional research is under way in determining whether the poorly utilized carbohydrates in this study could actually be well utilized over long incubation periods as a consequence of the formation of adaptive enzymes. Investigations on utilization of mixtures of carbohydrates and related compounds found in pecan tissues should also be made.

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