The effect of Oligosaccharides and their hydrolytic products on growth, sporulation and subsequent spore germination of Aspergillus flavus

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Introduction

Oligosaccharides are complex carbohydrates composed of two or more monosaccharide units united by glycosidic linkages that may differ in types (alpha, beta or mixed) and the carbon involved in the linkages. Most fungi first hydrolyze the oligosaccharides into simpler sugars before they are utilized by the micro-organisms. The ability of hydrolysis by the micro-organisms is linked with the formation of hydrolytic enzymes (Lilly and Barnett, 1953; Cochrane, 1958). A few fungi, however, are reported to utilize some oligosaccharides directly (Mandels, 1954; Lilly and Barnett, 1953; Wilson and Lilly, 1958), while in other cases, certain oligosaccharides appear to be utilized only if a second monosaccharide or other source of carbon is also present (Waters et al. 1953; Sistrom and Machlis, 1955; Machlis, 1957).

In an earlier study, it was found that *Aspergillus flavus* Link ex Fries yielded good growth and sporulation when the media contained different oligosaccharides as the carbon source (Grover and Bansal, 1968). In the present studies effort has been made to investigate whether *A. flavus* utilized the oligosaccharides directly or utilized their hydrolytic products. Besides, growth and spore germination of the organism was also observed.

Material and Methods

The isolate of *Aspergillus flavus* was the same as used by Grover (1964) and Grover and Bansal (1968). The basal medium consisted of KH_2PO_4 5 g; $MgSO_4.7H_2O$ 2.5 g; KNO_3 10 g; $FeCl_3$ 0.01 g; and distilled water to make 1 litre. The carbon sources were added to the basal medium in amounts calculated to give equivalent concentration of carbon. The methods for sterilization, inoculation, growth measurements and spore germination were the same as described by Grover and Bansal (1968). The residual carbon in the cultural filtrates was determined colorimetrically by the method described by Snell et al. (1961).

Results

Sucrose, lactose and raffinose were selected as the three oligosaccharides which were tested along with their hydrolytic products for the growth, sporulation and subsequent spore germination of *A. flavus*. The chromatographic analysis of the residual carbohydrates in the cultural filtrates containing different sugars after the growth of *A. flavus* have been given earlier (Grover and Bansal, 1968).

Sucrose and its Hydrolytic Products: — Sucrose, glucose, fructose and mixture of glucose and fructose (equal proportion) were added to the basal medium as the carbon sources and *A. flavus* was inoculated. The results are given in Table 1.

Table 1. Effect of hydrolytic products of Sucrose on growth, sporulation and subsequent spore germination of *Aspergillus flavus*

Carbon source [1]	Sporulation	Color of spores	Mycelial dry wt. (mg.) [2]	% spore germination	Carbon residue (mg. C/ml)
Sucrose	Abundant	Yellowish green	588	20.1	0
Glucose	Abundant	Deep green	748	36.2	0.63
Fructose	Abundant	Deep green	800	5.9	0.12
Glucose + Fructose	Abundant	Green	614	4.9	0.38

[1] Each flask of the set contained a total of 842 mg/100 ml. of carbon. The mixture of the carbon sources as well as individual carbon sources added in equel proportion.

[2] Average of three flasks containing 25 ml. of medium in each flask.

In all cases, the mycelial dry weight of the organism was good, though in case of media containing glucose or fructose it was better. While in the media containing sucrose alone or glucose + fructose, the growth was approximately equal (Table 1). The sporulation was abundant in all cases, though the mass coloration varied from yelowish green (sucrose), green (glucose + fructose) to deep green (glucose, fructose). Spores harvested from these different media, when germinated in water gave different responses. Germination was best in case of spores obtained from media containing glucose, while it was poor in case of fructose or glucose + fructose.

Lactose and its Hydrolytic Products: Lactose, glucose, galactose and mixture of glucose and galactose (equal proportion) were added to

the basal medium as the carbon sources and the results are given in Table 2.

Table 2. Effect of hydrolytic products of Lactose on growth, sporulation and subsequent spore germination of *Aspergillus flavus*

Carbon source [1]	Sporulation	Color of spores	Mycelial dry wt (mg.) [2]	% spore germination	Carbon residue (mg. C/ml)
Lactose	Poor	White	54	32.3	3.6
Glucose	Abundant	Deep green	748	36.2	—
Galactose	Fair	Green	553	6.8	4.0
Glucose + Galactose	Abundant	Deep green	596	5.7	2.32

[1] Each flask of the set contained a total of 842 mg/100 ml of carbon. The mixture of the carbon sources as well as individual carbon sources added in equal proportion.

[2] Average of three flasks containing 25 ml. of medium in each flask.

Very good growth was obtained when the media contained glucose, galactose or mixture of glucose and galactose, but very poor growth of the fungus was observed in case of lactose alone (Table 2). The sporulation was almost equal in all cases except in case of media containing lactose, where it was very poor. The mass spore coloration varied from white (lactose), green (galactose) to deep green (glucose and glucose + galactose). Spores harvested from these different media when germinated in water, gave different responses. Germination was best in case of spores obtained from media containing glucose or lactose, while it was very poor in case of galactose or glucose + galactose. With the increase in incubation period to 16 days the mycelial yield of the fungus was slightly increased and correspondingly the carbon residue in cultural filtrate decreased slightly.

Raffinose and its Hydrolytic Products: — The raffinose molecule contains the disaccharide structures of sucrose and melibiose. Complete hydrolysis yields equal amounts of glucose, fructose and galactose. All these sugars were used alone and in different combinations in the basal medium and the results are given in Table 3.

Very good growth was obtained when the media contained raffinose or fructose, followed by glucose or melibiose + sucrose (Table 3). Sucrose or galactose alone gave slightly better growth than melibiose alone. Sporulation, however, was very good in all cases except in case of media containing galactose, where it was relatively poor. The color of the spores varied from yellowish green to olive green. Subsequent

Table 3. Effect of hydrolytic products of Raffinose on growth, sporulation and subsequent spore germination of *Aspergillus flavus*

Carbon sources [1])	Sporulation	Color of spores	Mycelial dry wt. (mg.) [2])	% spore germination	Carbon residue (mg. C/ml)
Raffinose	Abundant	Olive green	859	29.5	0.98
Glucose	Abundant	Deep green	748	36.2	—
Fructose	Abundant	Deep green	800	5.9	—
Galactose	Fair	Green	533	6.8	—
Galactose + fructose + glucose	Abundant	Olive green	582	12.7	1.58
Sucrose	Abundant	Yellowish green	588	20.1	—
Melibiose	Abundant	Yellowish green	456	23.9	0
Melibiose + sucrose	Abundant	Yellowish green	662	9.6	0

[1]) Each flask of the set contained a total of 842 mg/100 ml of carbon. The mixtures of the carbon sources and the individual carbon sources added in equal proportion.

[2]) Average of three flasks containing 25 ml. of medium in each flask.

spore germination was best in case of glucose followed by raffinose, melibiose and sucrose. Germination was poor when spores were obtained from media containing fructose, galactose, melibiose + sucrose or glucose + galactose + fructose.

Discussion

The experimental evidence obtained in this study indicates that sucrose and raffinose are hydrolyzed by *Aspergillus flavus* during its gowth. Some of the sugars, especially sucrose, appeared to be completely hydrolyzed. Satisfactory evidence of the partial hydrolysis of raffinose was also obtained. Similar results have been reported by Govindarajan (1953) with yeast and Wilson and Lilly (1958) with *Ceratocystis coerulescens* and *Thielaviopsis basicola*. The low yield of mycelium obtained when the organism was cultured in melibiose and sucrose in the medium suggests that the chief source of utilizable carbon was fructose. On the other hand, higher yields of mycelium in the hydrolytic products of lactose both individually or in combination and poor mycelial growth in lactose, indicate that lactose was not hydrolyzed by *A. flavus*. What-

ever little growth was obtained in the presence of lactose is a result of its direct assimilation. Lilly and Barnett (1953) pointed out that many fungi which grew poorly on lactose, would grow better, if incubated for longer period. With *A. flavus*, the increase in incubation period did increase the mycelial dry weight slightly and correspondingly the residue in cultural filtrate also decreased sligthly. This probably means that synthesis of lactose is a limiting factor in the rate of growth of this fungus. The residual lactose and galactose individually in the cultural filtrates after 8 days incubation was found to be very high, whereas in case of glucose and fructose it was the least. The cultural filtrate of media containing raffinose had very little residue indicating thereby that it was not completely hydrolyzed. Cultural filtrate of media containing sucrose showed no carbon residue thus showing its complete hydrolysis.

The constituents of the medium influence directly internal reserves of the spores in fungi (Darby and Mandels, 1955; Grover, 1964; Allen, 1965; Sussman and Halvorson, 1966; Grover and Bansal 1968). The present findings reveal that when the substrate medium contained sucrose or raffinose, the spore reserves were normal. While when lactose was the carbon source in the medium, the reserves were slightly low as indicated by the germination of the spores. On media containing fructose or galactose, the spore germination was very poor indicating the lack of proper nutritional reserves. In case of mixture of glucose + fructose, glucose + galactose, melibiose + sucrose and glucose + galactose + fructose, the spore germination was rather poor. Sporulation, however, was not always related to growth rate or subsequent spore germination, since in the case of media containing lactose, although the fungal growth was low and sporulation was poor, yet the spore germination was normal. Whereas in contrast, in case of media containing fructose, the growth and sporulation was abundant, but spore germination was poor. The precise biochemical role of these sugars in influencing the internal reserves of the spores still needs to be determined.

Summary

Sucrose was completely hydrolyzed, raffinose was partly hydrolyzed, whereas lactose was not hydrolyzed by *Aspergillus flavus* during its growth. The hydrolysed products of these oligosaccharides, however, gave better growth and sporulation. Presence of lactose in the basal medium resulted in poor growth and poor sporulation. Spores harvested from media containing lactose, sucrose and raffinose gave normal germination in distilled water, whereas when the media contained simpler sugars like fructose or galactose, the spore germination was poor. Obviously the internal reserves of the spores remained insufficient in metabolites leading to the normal spore germination process.

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