

Effect of Alcohols and organic Acids on Growth, Sporulation and subsequent Spore Germination of *Aspergillus Flavus*

By R. D. Bansal and Rajendra K. Grover*)

Department of Botany and Plant Pathology, Punjab Agricultural University, Ludhiana, India

Introduction

Carbon is indispensable and occupies a dominant position amongst the essential elements that are required for the growth and sporulation of fungi (Lilly and Barnett, 1953; Cochrane, 1958). Among different carbon sources alcohols and particularly sugar alcohols, formed by the reduction of aldehyde and ketonic groups of the sugars, are important carbon sources for the growth and reproduction in fungi (Leben and Keitt, 1948; Brewer, 1960; Hawker, 1939; Subramanian, 1961; Perlman, 1965). On the other hand, organic acids have been reported to be poor sources of carbon for most fungi (Leben and Keitt, 1948; Dyal, 1960; Perlman, 1965).

In earlier studies with *Aspergillus flavus* Link ex Fries it was found that the organism utilized some of the carbohydrates including oligosaccharides directly or their hydrolytic products (Grover and Bansal, 1969; Bansal and Grover, 1969). In the present investigations, attempts have been made to study the effect of various alcohols and organic acids on growth, sporulation and subsequent spore germination in *A. flavus* and the results are reported herein.

Materials & Methods

The isolate of *Aspergillus flavus* was the same as used by Grover (1964), Grover and Bansal (1969), Bansal and Grover (1969). The basal medium, method of sterilization, growth characteristic and spore germination were the same as described earlier. The organic acid concentrations were based on the carbon sources calculated to give 8.42 mg/ml carbon in the media equivalent to 20 g/l of sucrose. In case of alcohols the carbon concentration was kept half of the given concentration, i. e. 4.21 mgC/ml, in order to avoid their toxicity (Strider and Winstead, 1960). The pH of the medium was maintained at 5.5.

Only pyrex glass-ware, analytical grade chemicals or B. D. H. chemicals were used throughout the investigations.

*) Present Address: Department of plant pathology, Agricultural University, Hissar, Haryana, India.

Results

Alcohols: Eight alcohols were used in the basal medium as the carbon source and the results are given in Table 1.

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

Carbon sources ¹⁾	Sporulation	Colour of spores	Mycelial dry wt. (mg) ²⁾	% spore germination ³⁾	Final pH
Methanol	Poor	Light green	24	18.6	5.2
Ethanol	Fair	Olive green	99	0	5.8
Isopropanol	Poor	Light green	8	16.4	5.0
Butanol	Poor	White	29	18.3	5.0
Glycerol	Abundant	Deep green	615	2.1	6.0
Sorbitol	Abundant	Deep green	598	1.0	6.0
D-mannitol	Abundant	Green	704	0.5	6.0
Dulcitol	Abundant	Green	435	1.7	6.0

¹⁾ In the first four alcohols, each flask of the set contained a total of 4.21 mg/ml of carbon and in the rest 8.42 mg/ml of carbon.

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

³⁾ Spores harvested from respective medium were germinated in distilled water. Data are average of 300 spore counts from 4 replicates.

Good growth was obtained when the media contained mannitol, glycerol, sorbitol, and dulcitol, while it was poor in other alcohols (Table 1). Sporulation was abundant in the media containing sugar alcohols and glycerol but was poor in most of the alcohols except in ethanol, wherein it was fair. The mass spore colouration varied from whitish to light-green, olive green, green to deep green. Spores harvested from these media, when germinated in water, gave variable response. Spore germination was found good in case of methanol, isopropanol or butanol, while spore germination was poor when spores were harvested from media containing glycerol, sorbitol, mannitol or dulcitol. Spores produced on media containing ethanol did not germinate.

Organic acids: Monocarboxylic, dicarboxylic and unsaturated organic acids were used as sole sources of carbon in the basal medium and the results are given in Table 2.

Fair growth was obtained when the media contained citric or succinic acids followed by malic or lactic acids while it was very poor in all the monocarboxylic and unsaturated organic acids; propionic acid did not yield any growth of the fungus. Sporulation was, however, abundant in malic acid and fair in succinic, citric and lactic acids, while in remaining organic acids no sporulation was observed. The spore colouration also varied from white to deep green. The spores

Table 2. Effect of organic acids on the growth, sporulation and subsequent spore germination of *Aspergillus flavus*

Organic acids ¹⁾	Sporulation	Colour of spores	Mycelial dry wt. (mg) ²⁾	% spores germination ³⁾	Final pH
Monocarboxylic acid					
Formic acid	Absent	—	4	—	5.2
Acetic acid	Absent	—	3	—	5.2
Propionic acid	Absent	—	0	—	5.4
Butyric acid	Absent	—	3	—	5.2
Dicarboxylic acid					
Succinic acid	Fair	Light green	101	0	6.0
Hydroxy acid					
Lactic acid	Fair	Light green	43	0	6.0
Malic acid	Abundant	Olive green	59	0	6.0
Citric acid	Fair	White	121	2.2	6.0
Unsaturated acid					
Maleic acid	Poor	White	5	10.1	5.2

¹⁾ Organic acids were added at the rate of 8.42 mgC/ml in the basal medium. Three replicate flasks were used, each containing 25 ml of the medium.

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

³⁾ Spores harvested from respective medium were germinated in distilled water. Data are average of 300 spore counts from 4 replicates

harvested from media containing different organic acids, when germinated in water, showed no germination except from maleic and citric acids, where it was very poor.

Discussion

The present investigations reveal that alcohols and organic acids were either utilized poorly or not at all by *Aspergillus flavus*. The sugar alcohols, however, supported relatively better growth of the organism. Subramanian (1961) reported that the maximum growth of *Fusarium udum* was obtained in the presence of glycerol followed by mannitol and dulcitol with an increased incubation period. In the present study, sugar alcohols and glycerol were utilized for growth and sporulation of the fungus. Similar results also have been reported by Wolf et al. (1950) with *Monosporium spiospermum*; Tandon and Aggarwal (1956) with *Gloeosporium psidii*, *G. citricolum* and *G. limetticum*; Perlman (1948) with *Memmoniella echinata* and *Stachybotrys atra*.

Alcohols are poor sources of carbon for the growth of *A. flavus*. Cochrane (1958) reported that *Aspergillus versicolor* grew well on ethanol and not on the other primary alcohols whereas alcohols with higher number of carbon atoms (C—12 to C—18) supported some growth

of the fungus. Page (1952) found that alcohols were inhibitory in action to *Pilobolus* sp.

Among the organic acids, succinic and citric acids followed by malic and lactic acids were relatively good sources of carbon for the growth of the present organism. It was seen that organic acids with more than one carboxylic group supported good growth- while monocarboxylic acids supported no growth of the present isolate. It became evident that the number of carboxylic groups in organic acids had a great influence on their utilization by this fungus. Hawker (1950) reported that the number of hydroxyl groups played a vital role in the utilization of an organic acid but as such these are poor sources of carbon for the growth of fungi (Leben and Keitt, 1948; Perlman, 1948; Page, 1952; Subramanian, 1961).

Carbon sources which support good growth may or may not be a good source of sporulation. Good sporulation was observed in media containing glycerol, dulcitol, sorbitol and mannitol. No sporulation was observed in alcohols. Sugar alcohols also have been reported to be poor carbon sources for sporulation in many fungi (Hawker, 1939; Steinberg, 1939).

Subsequent spore germination, however, was not related to sporulation and growth of the fungus (Grover, 1964; Grover and Bansal, 1969). Although sporulation was good in media containing glycerol, dulcitol, mannitol and sorbitol, but spores harvested from these media germinated poorly. Whereas in methanol, isopropanol, butanol, the spore germination was good though sporulation was very poor. While in case of organic acids the sporulation was absent except in maleic and citris acids. Obviously the contribution of alcohols and organic to the internal reserves of spores of *A. flavus* is very insignificant.

Summary

Dulcitol, sorbitol, mannitol and glycerol gave good growth and sporulation of *Aspergillus flavus*. Presence of methanol, butanol, isopropanol, or ethanol in the basal medium resulted in poor growth and sporulation. Spores harvested from media containing sugar alcohols and alcohols showed poor or no germination.

Citric, succinic, malic and lactic acids showed moderate growth and fair sporulation. There was some germination of spores harvested from media containing maleic and critic acids only.

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Autor(en)/Author(s): Bansal R. D., Grover Rajendra K.

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