Pak. J. Bot., 38(2): 443-448, 2006.

EFFECT OF VARIOUS CONCENTRATIONS OF GLUCOSE, DI-AMMONIUM TARTRATE AND CARROT EXTRACT ON THE GROWTH OF *ASPERGILLUS VARIECOLOR*

NASEEM F. USMANI, TOOBA HAQ AND FATIMA BI

PSCIR Laboratories Complex,
Off University Road, Karachi, Pakistan

Abstract

The effect of varying concentrations of glucose, di-ammonium tartrate and carrot extract on the growth and yield of metabolic products of *Aspergillus variecolor* has been examined. Media containing 10% glucose, 0.43% di-ammonium tartrate and 400g carrot extract per liter broth increased the mycelial growth by 50% which further resulted in an increase in by 36.8% yield of crude xanthone and 29.5% manitol the metabolic products of *Aspergillus variecolor*.

Introduction

The utilization of carbon is one of the most important element which occupies a unique position in the metabolic pathway of fungal metabolism. On the other hand the adequate source of nitrogen also plays an important role for good mycelial growth of fungi (Zamir & Hussain, 1970).

Considerable work has already been carried out on the nutrition of fungi (Fothergill & Jone.1958). Many species of fungi are specific in their nutritional requirements while using glucose as the carbon source with different nitrogen sources. Moreover it was also observed that the mixture of some aminoacids (lysine, arginin, histidine and asperginin) and carbohydrates as a source of nitrogen and carbon, support the better growth of fungi eg., *Curvularia lunata* and *Fusarium sulphurecum* (Anwar & Husain, 1983; Shahid & Zamir, 1971). Studies carried out using *Aspergillus variecolor*, *Penicillium chrysogenum* and *Penicillium palitans* by using the substrate of cellulosic constituents showed production of specific volatile metabolic compounds (Wilkins & Larsen, 2003). The present work was carried out under the specific conditions of pH and temperature for the growth of *Aspergillus variecolor* for its spore germination using different culture media. During the course of studies the standard Czapek Dox liquid medium has been modified, using different strengths of glucose and diammonium tartrate as carbon and nitrogen source, enriched with carrot extract as substrate, found to be more effective on mycelial growth (*Aspergillus variecolor*) with enhanced metabolic production (Lilly & Barnett, 1955).

The powdered mycelial mat when extracted with ethanol and pet-ether, (as solvent system) gave crystalline metabolic product, which were chemically evaluated and identified as Terrein and Kojic acid, previously isolated as metabolite of *A. variecolor*.

Material and Method

Based on the previous report (Qureshi *et al*., 1968), the fungal strain of *Aspergillus variecolor* (CMI–112543) was selected due to its metabolic products like Terrein of therapeutic and pharmacological importance (Gupta & Vishwanath 1955). The culture

*Corresponding author:

was maintained on Czapek Dox culture medium. The 7-days old sporulating fungal culture was then suspended in 50 ml flask containing sterilized distilled water and was shaken vigorously an in automatic shaker for 20 to 30 minutes. The spore suspension was then used as inoculum for the liquid culture medium in 100ml per 500ml flask in triplicate. The composition of the standard medium used was glucose 50.0, KH_2PO_4 1.0, KCl 0.5, $MgSO_4$ 0.5, $FeSO_4$ 0.01, g/l enriched with aqueous carrot extract, while the experimental medium "A" with varied concentration of glucose of 1.0, 5.0, 10.0 and 15.0% and the other contents of the Czapek Dox medium remained unchanged. Similarly, di-ammonium-tartrate and the carrot extract were used with various concentrations at the level of best-suited parameter to get the maximum growth of *Aspergillus variecolor* (Table 1).

Batch study: In the typical batch of 5 (1.0 liter) conical flasks and each containing 340 ml of the above mentioned medium "B" were prepared and autoclaved at 10 psi for 20 minutes. On the other hand, the separately prepared solution of di-ammonium tartrate solution of 7.56% and 15% strength were sterilized and the 10 ml of it was poured aseptically to each of the prepared culture flask at pH 4.8 prior to inoculation.

In each experiment 100 ml medium in 500 ml flasks was used in triplicates. The flasks were inoculated with 4.0mm discs cut from the growing edges of 7 days old culture of *Aspergillus variecolor* and incubated at 27°C for two weeks. After the incubation, the contents of the flasks were filtered, mycelium separated, washed and dried in an oven to constant weight at 55ºC, on previously weighed filter papers. The difference between the initial and final weight of filter papers gave the dry weight of mycelium. The average dry weights of the mycelium obtained were plotted against respective concentrations of nutritional factors used.

Terrein was isolated after 21 days of incubation from broth by extracting with ethyl acetate while, the xanthones were isolated from the dried powdered mycelium using soxhlet extraction method with petroleum ether and the mycelium was finally extracted methanol to isolate mannitol as reported by Kamal *et al*., (1970). Recently, the biosynthesis of Tajixanthone, Shamixanthone and related metabolites of *A. variecolor* were studied by incorporating experiments with simple and advanced precursors labeled isotopes (Ahmed *et al*., 1992)

Samples of both were withdrawn periodically to check sugar conversion by measuring the optical rotation using polarimeter (Table 2). Terrain was detected using thin layer chromatography technique on Kieselghur-60 with solvent system of petroleum ether, ethyl acetate (0.1: 4.9).

Results

In the first set of experiments it was observed that the weight of mycelium increased due to the increase in concentration of glucose upto 10%, whereas, 15% and 20% concentration showed retarding effect on mycelial production (Fig. 1). This may be due to the effect of osmotic pressure on cell contents. Similarly addition of 4.32 g/l of di-ammonium tartrate (DAT) with 10% glucose concentration further increased the yield of mycelium as compared to lower concentration of 1.08 g/l and 2.16 g/l DAT which gave low yield of mycelium. Like glucose further increase in the amount of DAT to 5.7 g/l did not enhance mycelial growth, in fact the growth was reduced appreciably (Fig. 2). This range was, therefore, selected as the optimum nitrogen requirement for the fungus.

Table 1. Chemical composition of medium 'A' and medium 'B'.

Composition	Medium "A"	Medium "B"
$C_6 H_{12}O_6$	5%	10%
KH_2PO_4	0.1%	0.1%
KCl.	0.05%	0.05%
$FeSO_4.7H_2O$	0.001%	0.001%
$MgSO_4.7H_2O$	0.05%	0.05%
$(CH(OH)CO_2NH_4)_2$	0.216%	0.43%
Carrot extract	200 g/l	400 g/l
pH	4.5%	4.5%

Table 2. Periodic determination of glucose consumption during mycelial growth.

Days of incubation	pH	Angle of rotation	Glucose%: (Consumed)
1st	4.5	1.05	5.0
6th	5.5	0.75	3.5
9th	5.9	0.58	2.7
12th	6.4	0.34	1.6
16th	7.0	0.20	0.95
21st	7.4	0.001	0.001

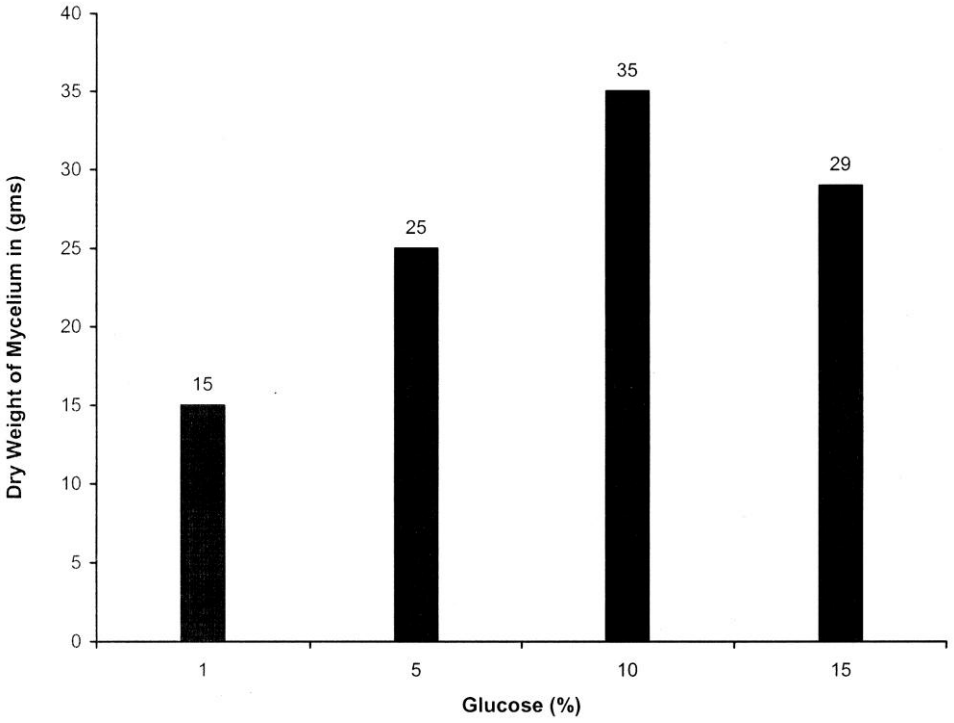

Fig. 1. Effect of different concentration of glucose on mycelium production of *Aspergillus variecolor*.

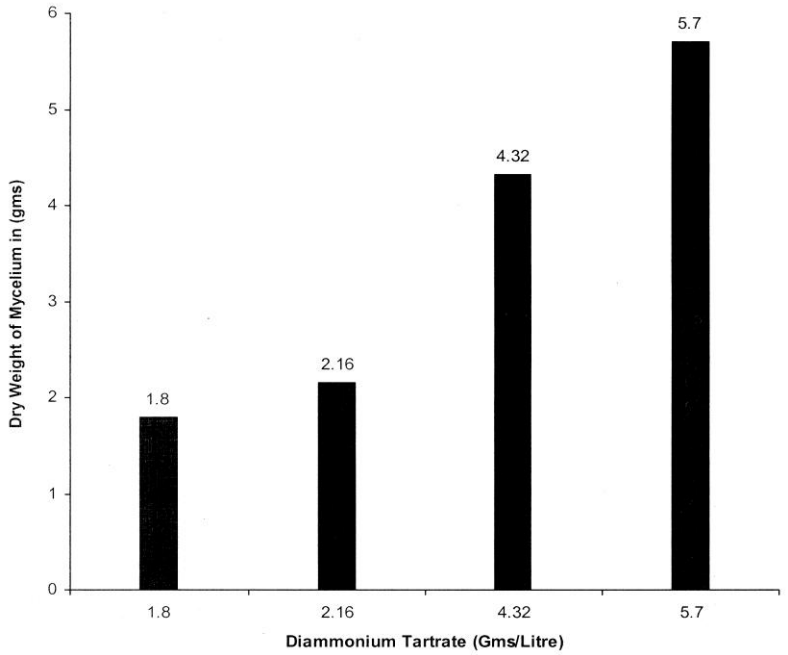

Fig. 2. Effect of different concentration of diammonium tartrate on mycelium production of *Aspergillus variecolor*.

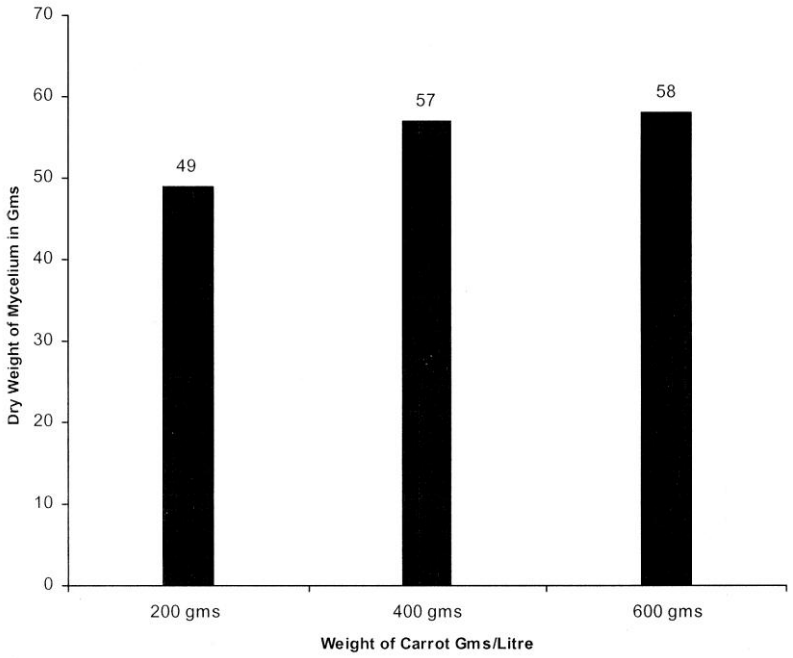

Fig. 3. Effect of different concentration of carrot extract on mycelium production *Aspergillus variecolor*.

Likewise, both 400 and 600 g. of carrot extract when incorporated with 10% glucose and 4.32 g/l DAT solution (0.43%) showed 58g and 59.6 g of mycelium, respectively. In the absence of carrot extract, however, yield of mycelium was effectively lower (Fig. 3). On the other hand, increasing the amount of carrot extract (400–600g) did not affect the growth appreciably and hence, in further study 400g carrot extract was selected for the use of further experiments.

Discussion

On the basis of experimental results related to nutritional requirements of *Aspergillus variecolor* using Czapek Dox culture medium has been modified accordingly. While using the various concentrations of glucose as carbon source and diammunium tartrate as nitrogen source, enriched with different volume of carrot extract, two comparative experimental media "A" and "B" were designed and developed as the best suited medium for the growth of *Aspergillus variecolor*.

The medium A which had 5.0% glucose concentration, formation of Terrein was initiated from 8th day of incubation period, whereas, the total sugar was consumed within 17 days. However, with medium B having 10% glucose, the time of Terrein formation delayed i.e, instead of 8th day, Terrein was detected on 11th day and sugar was completely utilized within 21 days. This may be due to extension in lag phase of growth. The fungus *Aspergillus variecolor* was adapted to lower concentration of chemicals and some times adjust itself to the new environmental condition before its multiplication starting metabolic activities. Besides the higher sugar level, *Aspergillus variecolor* took longer time for its complete utilization. Medium "B" produced 50% higher weight of mycelial growth than did media "A". This increase in weight of mycelium subsequently increased the yield of xanthones by 36.8% and mannitol by 29.5%, though the yield of Terrein was reduced by 30.7% Table 3. On the basis of above results a composition of the medium "B", proved to be the most suitable nutritive medium for obtaining maximum yield of mycelium with good content of metabolic products.

Table 3. Comparative results of media "A" and media "B" related to metabolic production.

Parametric studies	Media "A"(%)	Media "B"(%)
Weight of Mycelium/l	2.857	5.8
Time of metabolic production (Incubation period)	08(days)	11(days)
Sugar consumed	17(days)	21(days)
Yield of crude terrine/l	0.13	0.09
Yield of crude xanthone/l	0.048	0.076
Yield of crude mannitol/l	0.03	0.05

References

Ahmed, S.A., Bardshiri, E.S. Fadiar. 1992. Biosynthetic studies on Tajixanthone and Shamixanthone, polyketide hemiterpenoid metabolites of *Aspergillus variecolor*. *Aust. J. Chem*., 45(1): pp. 249-274.

Anwar, N. and S.S. Husain. 1983. Studies on nutrition of funi. part viii. effect on different amino acids and their combination on the growth of different fungi. *Pak. J. Sci. Ind. Res*., 26(4): 247.

Gupta, K.C. and R. Vishwanath. 1995. Anti tuberculous substances from *Aspergillus proliferans* and *Aspergillus veriecolor*. *Antibiotic and Chemotherapy*. 5: 496-498.

Kamal, A. Shaheen A. Husain, Radia Noorani, Mutraza, I.H. Qureshi, A.A. Qureshi. 1970. Studies in the biochemistry of microorganisms xi. Isolation of tajixanthone, shamixanthone, shaheenxanthone and mannitol from mycelium of Aspergillus stellatus, Curzi. *Pak. J. Sci. Ind. Res*., 13: 231.

Lilly, V.G. and H.I. Barnett. 1951. Physiology of fungi. McGraw Hill Book Company Inc.

Forthergill, P.G. and M. J. Jone. 1958. *Gen. Microbiology*, pp. 298.

Qureshi, I.H., A. Kamal, R. Noorani and S.A. Husain. 1968. Studies in the biochemistry of microorganisms. Part vii. Terrein and kojic acid, metabolic products of *Aspergillus stellatus* Curzi. *Pak. J. Sci. Ind. Res*., 4: 367.

Shahid, S.H and K. Zamir. 1971. Studies on the nutrition of fungi. VI. Effect of different combination of mono, di, trisaccharide on the growth of some fungi. *Pak. J. Sci. Ind. Res*., 3(1&2): 79-82.

Wilkins, Ken, Larsen and Kjeld Mirela. 2003. Volatile metabolites from in door molds grown on media containing wood constituents. *Environmental Sci. and Pollution Res*., 10(4): 206-208.

Zamir, K. and S.S. Husain. 1970. Studies on the nutrition of fungi IV. Effect of different nitrogen and carbon sources on the growth of six imperfect fungi. *Pak. J. Sci. Ind. Res*., 7: 171.

(Received for publication 2 July 2005)