30 minutes. The cold clear solution on neutralisation with dil. HCl gave 1·12 g (70%) of X*a*. It was crystallized from 40% ethanol, m.p. 230–31° (d). IR : 1730, 1690, 1585.

Similarly prepared were X*b* and *Xc*.

3,3'-Dimethylfurano (6,7-*d*)-1,2-*benzisoxazole* (*XIa*)

A mixture of phenoxy acetic acid (X*a*, 0·8 g), acetic anhydride (8 ml) and fused sodium acetate (1 g) was heated on an oil-bath at 160–170° for about 4 hr. The cold reaction mixture was poured over ice-water and worked up as usual to give 0·470 g (70%) of XI*a*. It was crystallized from ethanol, m.p. 139–40°; IR : 1625, 1570, 1075, 845; PMR (CCl_4) : 2·60 (*s*, 6H, 2×CH_3) and 7·53 (*s*, 2H, Ar–H at C–4 and C–2').

XI*b* and XI*c* were prepared as discussed above.

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VARIATIONS IN THE CELLULAR CONSTITUENTS OF *ASPERGILLUS NIDULANS* GROWN ON DIFFERENT CARBON SOURCES

R. SATHIAGANA SEELAN, S. MALATHI AND E. R. B. SHANMUGASUNDARAM
University Biochemical Laboratories, University of Madras, Guindy, Madras 600 025

ABSTRACT

Aspergillus nidulans was grown on six different sugars—glucose, fructose, sucrose, maltose, lactose and galactose; maximum growth was observed in glucose and fructose while lactose and galactose were poor carbon sources. Growth on lactose and galactose revealed marked variations in the levels of various cellular constituents when compared to growth on other sugars. Growth on lactose, particularly, showed significant decrease in the total carbohydrate, glycogen and lipid content, and pronounced increase in nucleic acids (DNA and RNA) levels; growth in this sugar also implied cell shrinkage.

INTRODUCTION

THE utilization of sugars by *Aspergillus nidulans* has been studied in detail by Agnihotri[1–4], Mehrotra and Agnihotri[5,6] and Roberts[7]. Similar studies have also been conducted in other microorganisms such as the *Candida* group[8], *Clostridium tetani*[9], *Mycoplasma agalactiae*[10], *Aspergillus niger*[11] and *Penicillium digitatum*[12]. However, no report has so far appeared regarding the changes observed in the cellular constituents of *A. nidulans* grown with different carbon sources in the medium. The results of such study are presented in this paper.

MATERIALS AND METHODS

Organism : A wild strain of *A. nidulans* with green conidia (Glasgow stock of strain) was used

Preparation of cultures: Media were prepared according to the method of Pontecorvo *et al.*[13], containing 10 g/l of the carbon source. The media (50 ml) were dispersed into 250 ml Erlenmeyer flasks and sterilized in an autoclave at 15 lb/sq. inch for 15 min. The flasks were inoculated with a heavy conidial suspension of *A. nidulans* (containing approx. 10^6 spores/ml in sterile saline) and incubated at 37° C for 4 days.

Determination of cellular constituents

The following were determined in the dried mycelia.

(*a*) *Carbohydrates*: Dried mycelium (50–100 mg) was extracted with 60% KOH by heating in a water-bath for 15 min. This was centrifuged and the residue was washed thrice with 33% KOH solution and the supernatant fractions were pooled and diluted to 33% KOH final concentration. Total alkali soluble carbohydrates were determined in this fraction by the Anthrone method[14]. Glycogen was precipitated from the alkali soluble carbohydrate fraction by the method described by Morales *et al.*[15] and then determined by the Anthrone method[14].

(*b*) *Protein*: Protein content was determined by the method of Lowry *et al.*[16].

(*c*) *Nucleic acids*: Dried mycelia (50–100 mg) were homogenized in distilled water (5·0 ml) using a mortar and pestle. 10% TCA (5 ml, ice-cold) was added and kept in the cold for 30 min. The mixture was centrifuged and the precipitate was subsequently washed thrice with ice-cold 10% TCA and then treated with 10 ml of 95% ethanol. The lipid-free precipitate was resuspended in 5·0 ml of 5% TCA and kept in a water-bath maintained at 90° C for 15 min., with occasional stirring. After centrifugation, the supernatant was used for the determination of DNA[17] and RNA[18].

(*d*) *Lipids*: Total lipids were extracted by the method of Folch *et al.*[19] using $CHCl_3 : CH_3OH$ mixture. The lipid contents were dissolved in $CHCl_3 : CH_3OH$ mixture and aliquots were used for the determination of triglycerides[20], sterols[21] and phospholipids. Phospholipids were determined by the method of Fiske and Subbarow[22] as inorganic phosphate after digestion with $HClO_4$.

Results and Discussion

Earlier studies on the utilization of sugars in *A. nidulans*[1–6] and our studies (Table I) confirm the fact that glucose and fructose are the best carbon sources for growth. Galactose and lactose are poor carbon sources and growth on sucrose and maltose is intermediate.

Table I

Dry weights of mycelia of A. nidulans *grown on different carbon sources*

Values are expressed as Mean ± S.D. (5 samples)

Carbon source	Dry wt. of mycelia (mg/50 ml culture)
Glucose	242·0 ± 12·4
Fructose	226 2 ± 18·0
Sucrose	197·3 ± 11·3**
Maltose	160·1 ± 11·0*
Lactose	112·0 ± 12·0*
Galactose	52·4 ± 5·2*

Statistically significant variations (compared to glucose as carbon source) are indicated by $*p < 0.001$, $** p > 0.01$.

Among the various sugars studied, galactose and lactose cause marked variations in the levels of various cellular constituents (Tables II and III). Lactose is a better carbon source than galactose (Table I). A study of the cellular constituents in galactose and lactose grown mycelia reveal similar changes, but striking differences exist when these variations are compared to that grown on glucose and other sugars.

There is a pronounced decrease in the alkali extractable carbohydrate (AEC) and glycogen levels in lactose (35% and 38% decreases, respectively) and galactose (60% and 67% decreases, respectively) grown mycelia (Table II); sucrose and maltose grown mycelia show significant decrease in AEC level and less significant decrease in glycogen content. Mycelia grown on fructose exhibit no significant changes. The decrease in the content of glycogen when the organism was grown on various sugars was in the order of glucose, fructose, maltose, sucrose, lactose and galactose.

The decrease in glycogen content in cultures grown on lactose and galactose may be due, either to the increased glycogenolysis or to decreased glycogen synthesis. Similar observations have been noticed in animal tissues also. Studies conducted in rats by Otomo[23] have revealed that glycogen production in liver by oral or intravenous injection of sugars decreased in the following order of effectiveness: glucose, fructose, galactose, sucrose, maltose and lactose. Fishoff and Devel[24] observed that rats fed on purified rations of lactose or beta-lactose failed to survive, when compared to those fed on a diet containing galactose, sucrose or glucose.

TABLE II

Alkali extractable carbohydrate, glycogen, protein and nucleic acid levels in A. nidulans *grown on different carbon sources*

Values are expressed as Mean ± S.D. (5 samples)

Carbon source	Alkali extractable carbohydrate	Glycogen	Protein	DNA	RNA	RNA/DNA ratio
	expressed as % dry mycelia					
Glucose	41·6±1·4	24·7±3·0	7·06±0·69	0·26±0·02	0·77±0·07	2·96
Fructose	37·0±4·4[a]	24·4±2·1	3·50±0·58*	0·41±0·02*	0·80±0·05	1·93
Sucrose	27·5±3·2*	19·2±1·9[a]	4·94±0·13**	0·37±0·06[a]	0·57±0·01**	1·54
Maltose	30·1±0·8*	21·9±0·7[a]	5·31±0·24**	0·30±0·07	1·21±0·20**	3·96
Lactose	27·1±0·1*	15·5±0·5**	6·51±0·12	0·52±0·03*	2·38±0·12*	4·61
Galactose	17·4±3·0*	8·5±0·7*	5·18±0·16**	0·31±0·01**	1·80±0·12*	5·73

Statistically significant variations (compared to glucose as carbon source) are indicated by $^{*}p < 0{\cdot}001$; $^{**}p < 0{\cdot}01$; $^{a}p < 0{\cdot}02$; $^{a}p < 0{\cdot}1$.

TABLE III

Lipid content in A. nidulans *grown on glucose and lactose as carbon sources*

Values are expressed as Mean ± S.D. (5 samples)

Lipid components	Glucose media (% dry mycelia)	Lactose media (% dry mycelia)
Total lipids	9·66±0·68	6·66±0·37*
Sterols	0·28±0·04	0·16±0·01**
Phospholipids	1·67±0·03	1·59±0·05[b]
Triglycerides	3·31±0·06	2·29±0·06[b]

Statistically significant variations (compared to glucose media) are indicated by $^{*}p < 0{\cdot}001$; $^{**}p < 0{\cdot}01$; $^{b}p < 0{\cdot}05$.

The DNA content (% dry mycelia) is significantly increased in lactose (100% increase) media. Slightly elevated DNA levels are observed in fructose (57% increase), sucrose (38% increase), maltose (15% increase) and galactose (17% increase). The RNA levels are also significantly elevated in lactose (200% increase), galactose (130% increase) and maltose (56% increase) media. A decrease in RNA level is observed in sucrose media alone. Elevations in RNA/DNA ratio are observed in galactose and lactose media; maltose medium exhibits a slight increase while media containing fructose and sucrose show a decrease. Protein content in lactose media is not significantly lowered (Table II); other sugars show significant reduction in protein content when compared to glucose media. Increase in DNA content (Table II) may be attributed to a greater rate of cell division. This increase may, therefore, result in a greater number of cells being present in the mycelia. Thus the growth of *A. nidulans* in carbon sources other than glucose seems to result in varying levels of cell shrinkage, the shrinkage being maximum in lactose medium. The RNA/DNA ratio indicates increased protein turnover in lactose and galactose media (Table II), which may probably be due to the induction of the various enzymes associated with lactose and galactose metabolism.

Since significant variations were observed in the cellular constituents of lactose grown mycelia, lipid components were also examined when this sugar was used as a carbon source (Table III). In lactose medium, significant decrease in the total lipids and lipid component levels were observed. The reduction could be due either to the increased lipolysis or decreased lipogenesis. Studies on the synthesis of fats in *A. nidulans* undertaken by Garrido and Walker[25] have shown that xylose, glucose and maltose are the best substrates for the production of fat.

Since the carbon constituents of the growth medium and the physiological state of the cells affect the relative extent of utilization of the EMP and HMP glycolytic pathways, the observed variations may be better understood if a study on the activities of various

enzymes associated with carbohydrate metabolism is made.

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Further information may be had from : Dr. Mrs. Sumati V. Bhide, Cancer Research Institute, Tata Memorial Centre, Parel, Bombay 400 012.

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Oil Technologists' Association of India, Southern Zone, Hyderabad, is organising the 36th Annual Convention and a Symposium on "Processing of Oilseeds, Oils, Byproducts and Derived Products, Techno-Economic Aspects" on Saturday and Sunday, February 14th and 15th, 1981 at the Regional Research Laboratory, Hyderabad 500009, India.

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