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LIPID STUDIES ON *CANDIDA BOIDINII* GROWN ON METHANOL

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The carbon source for aerobically grown culture of *Candida boidinii* influenced the lipid content and the fatty acid composition of the lipid components. Effects of nitrogen source were observed with different concentrations of ammonium sulphate. Cells grown in glucose and methanol in the presence of ammonia nitrogen respectively produced 22.1 % and 12.0 % total lipids. TLC results revealed that the major components of the total lipids were phospholipids (58-60 %). Among the non polar lipids the major components were triglycerides and free fatty acids. The GLC analysis showed that the lipid principally consisted of unsaturated acids with both carbon sources.

Key words: Candida boidinii; Phospholipids; Triglycerdies; Ammonium sulphate; Methanol.

INTRODUCTION

In recent years interest in microbial lipids has been renewed because of an urgent need for prudent utilization of alternative renewable resources as a carbon source for production of lipids and because of some new practical applications of microbial lipids. Therefore, a variety of substrates were considered for the growth of oleaginous organisms, covering almost all the available forms of carbohydrates.

A major carbon source is the alkane feed stock, easily obtained from the petroleum industry and consumeable micro-organisms. Alkanes as a feed stock for production of single cell proteins (SCP) has been extensively studied particularly in Britain [1]. Methanol has now emerged as a challanger because of its ready availability. ICI is using a fermenter with a capacity of 1.5 x 10^6 litres for the production of single cell protein from methanol.

It has, however, been generally recognized that variations for substrate and growth conditions influence the lipid composition [3]. In the present study methanol assimilating yeasts have been examined with a view to know their ability of lipid production. The need for this enquiry arose essentially from the fact that information on lipid components of the methanol assimilating yeasts is less readily available. The knowledge thus obtained could become helpful in establishing parameters for the commercial production of lipids using methanol as a feed stock. However, growth in presence of ethanol has shown to cause changes in the lipid composition of many organisms [4].

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A methanol utilising yeast, *Candida boidinii* first isolated in Japan, has been studied for its cultivation. Both glucose and methanol have been used and compared as the carbon sources for the growth of *Candida boidinii* and its lipid production.

MATERIALS AND METHODS

Methanol utilising yeast *Candida boidinii* was used throughout the study. It was obtained from (NCYC) National Collection of Yeast Cultures, Food Research Institute Norwich. The yeast was maintained on slant cultures of the following medium.

$(NH_4)_2SO_4$, 2.0g; KH_2PO_4, 1.5g; $MgSO_4$ $7H_2O$, 0.5g; $CaCl_2$ $2H_2O$ 30 mg; $MnCl_2$ $4H_2O$, 5.0 mg; $ZnSO_4$ $7H_2O$, 5mg; Cu SO_4 $5H_2O$, 0.5mg; biotin, 2 μg; thiamin HCl, 200 μg and 7.5 gm of methanol was added per litre of the medium to give approximately the same concentration of carbon source as that of glucose. Initial pH was adjusted to 5.6. Cells were precultured aerobically for 2-3 days at 30° in 500 ml. flasks containing 100 ml. of growth medium and 10 ml. of culture broth were transferred to 1000 ml. flasks containing 300 ml. of medium.

Main cultivation was performed on a rotary shaker at 30°. Cell growth was followed spectrophotometrically at 660 nm. The cells were harvested by centrifugation for 10 minutes at 3000 rpm. Procedures for the determination of dry cell weight and for the extraction of total lipids from dried cells by chloroform/methanol/mixture were the same as described previously [5].

Neutral lipids and phospholipids were separated by TLC on pre-coated silica gel plate of 20 cm x 20 cm x 0.25

mm dimensions. Phospholipids were further separated by TLC with chloroform-methanol-ammonia (30 %) and water (60:35:5:2.5) mixture. Identification of lipids was made with known standards after coloration with iodine vapours. The principal components were identified by composition of R_f values and by their reactions with various reagents.

Determination of fatty acids by gas chromatography. The extracted lipids were saponified with 0.5N alcoholic potassium hydroxide in the usual manner [6]. The soap solutions were freed from the non-saponifiable fraction and acidified with 4N sulphuric acid. The identity and percentage of the fatty acids was determined by the gas chromatographic analysis with the column PEGS at 210°. Injector and detector temperatures were 220° and 250° respectively. Nitrogen was used as carrier gas with a flow rate of 40 ml. per minute.

RESULTS

Effects of carbon source on growth and lipid contents. Table-1 shows the results of studies with glucose and methanol as carbon sources. The dry wt. of cells as well as yield of lipids on dry wt. basis have been recorded. Methanol concentrations of 1 % level showed the optimum growth which decreased by increasing the concentration of methanol and ultimately almost sized at 5 % level.

Effect of nitrogen concentration on lipid and its composition. Table-2 shows the results with various nitrogen concentrations in the media on the lipid contents. A considerable difference in the amount of extractable total lipids was observed with a step-wise increase in ammonium sulphate.

The fatty acid profiles from cells grown on glucose or methanol as the sole carbon source were comparatively examined as factor of nitrogen concentration (Table 3).

With the increase of ammonium sulphate concentration from 0.006 to 0.075 % a decrease in the amounts of 18:1 and an increase in the amounts of 18:2 acids was observed in case of both the carbon sources. More striking changes were found by increasing ammonium sulphate from 0.025-0.075 (Table 3), e.g. the amount of 18:1 acids in methanol grown cells decreased remarkably from 32.5 % to 23.4 %.

Effect of methanol on fatty acid composition. The lipids so obtained with both carbon sources were separately evaluated for their fatty acid composition.

Table 3 shows the fatty acid composition of the main lipid components from cells grown on glucose and methanol as carbon sources respectively. Concentration of methanol as well as ammonium sulphate was varied. The lipids were characterized by higher percentages of unsaturated fatty acids in the methanol grown cells than with the glucose grown cells. The fatty acid composition of the lipids indicated that 16.0, 16:1, 18:1 and 18:2 were the predominant fatty acids with considerable amount of 18:0 and 18:3 in glucose grown cells. The quantity of 18:2 acid during growth on glucose markedly decreased in

Table 1. Cell dry wt. and lipid contents of *C.boidinii* cells.

pH	Methanol %	Glucose %	Dry wt. mg/l	Lipid % dry wt.
4.0	1	–	570	10.3
5.0	1	–	666	12.0
5.0	4	–	490	9.0
4.0	–	2	1112	20.0
5.0	–	2	1239	22.3

Table 2. Effects of nitrogen concentration on lipid production of *C.boidinii.*

Ammonium sulphate %	Carbon sources	Dry wt. mg/l	Total lipids % dry wt.
0.006	Glucose	1145	20.6
	Methanol	730	12.0
0.013	Glucose	1223	22.0
	Methanol	677	12.2
0.025	Glucose	1239	22.3
	Methanol	666	12.0
0.050	Glucose	1280	10.0
	Methanol	695	7.30
0.075	Glucose	1350	6.8
	Methanol	728	5.8

Table 3. Fatty acid composition of *Candida boidinni* with different carbon sources and different nitrogen concentrations.

Ammonium sulphate % w/w	Carbon source	Fatty acids						
		16:0	16:1	18:0	18:1	18:2	18:3	Unsaturated acids (%)
0.006	Glucose	20.0	16.0	8.5	14.7	37.0	T	67.7
	Methanol	15.8	25.1	5.7	42.2	6.4	T	73.7
0.013	Glucose	20.0	16.0	7.4	15.0	37.0	T	68.0
	Methanol	17.0	26.5	6.0	40.0	7.0	T	73.6
0.025	Glucose	19.0	19.0	5.0	10.9	41.3	T	72.1
	Methanol	19.0	27.0	3.1	32.5	12.4	0.4	73.1
0.075	Glucose	17.9	21.7	4.8	7.2	45.1	0.2	74.2
	Methanol	20.0	31.5	2.2	23.4	17.1	0.8	72.8

cells grown on 1 % methanol accompanied by an increase in 16:1 and 18:1 acids.

The general composition of the non-polar lipid components as revealed by thin layer chromatography shows that the major component was free fatty acids and triglycerides. Triglycerides and sterol esters were 5.7 % and 2 % respectively.

Phospholipids were a major component of total lipids making 58-60 % of the total lipid grown on either 1 % methanol or 2 % glucose. The composition of the lipids did not change significantly when grown on 4 % methanol. The cell yield and total lipids were however low.

The analysis of the phospholipid composition showed that the major phospholipid was phosphatidylcholine (49 %) in cells grown on 2 % glucose or 1 % methanol. But the cells grown on 4 % methanol resulted in lower phosphatidylcholine (PC) with an increase in the phosphatidylethanolamine (PE) Phosphatidylserine (PS). Phosphatidylinositol (PI) and phosphatidic acid components.

DISCUSSION

C. boidinii cells grown on 2 % glucose and 1 % methanol with 0.075 % ammonium sulphate showed a considerable difference in the amounts of total extracted lipids. A remarkable increase of lipid contents with 0.025 % of ammonium sulphate was observed. Ammonium sulphate 0.025 % seems to be the optimum concentration for the maximum lipid production because a remarkable lipid accumulation generally occurs under the nitrogen limitation conditions [7-8]. A decrease in lipid content was observed with methanol as compared to glucose which reflects its decreased ability to utilise methanol. It is, therefore, inferred that *Candida boidinii* is unable to utilise methanol as efficiently as glucose. Results showing that methanol at 5 % level is toxic and prevents growth [9] have already been reported.

C. boidinii can be classified as a medium lipid producer. The general level of lipids (12 % on dry wt. basis) observed on methanol suggests that lipid is not the primary energy storage form in *C.boidinii.* This view is confirmed from its lipid composition analysis showing that major lipid component is phospholipids and not triacylglycerol which are usually the major components of stored lipids.

The observed decrease in cell contents while using methanol may reflect a decreased ability to utilize methanol. It seems that the growth on methanol follows the pattern as observed on higher alcohols [10]. Although these effects on growth are completely and rapidly reversible, showing that the initial growth of the cells transferred to a medium without alcohol, is restored after a short period, the duration of which depends on the concentration of alcohols. After incubation with methanol at a low concentration, the growth rate was restored before any change in lipids could be measured. It is thus observed that alcohols act also on easily reversible molecular interactions [11].

The present studies support results obtained earlier in some methanol utilizing bacteria showing that methanol can produce lipids with unsaturated fatty acids as effectively as glucose although the yield of total lipids is about 50 % in methanol (12.0 %) compared to glucose (22.3 %). Such effects have already been observed in some methanol utilizing bacteria [12].

The fatty acid composition of yeast on methanol did not show accumulation of marked quantities of odd chain fatty acids. The amount of 18:1 acids in methanol grown cells remarkably decreased while those of 18:2 increased with increasing ammonium sulphate concentrations in the medium. Interestingly there is a reverse tendency between two carbon sources in the amounts of 16:0 and total unsaturated acids, 16:0 acid was decreasing in glucose grown cells and increasing in methanol grown cells. In reverse total unsaturated acids were increasing in glucose grown cells and decreasing in methanol grown cells.

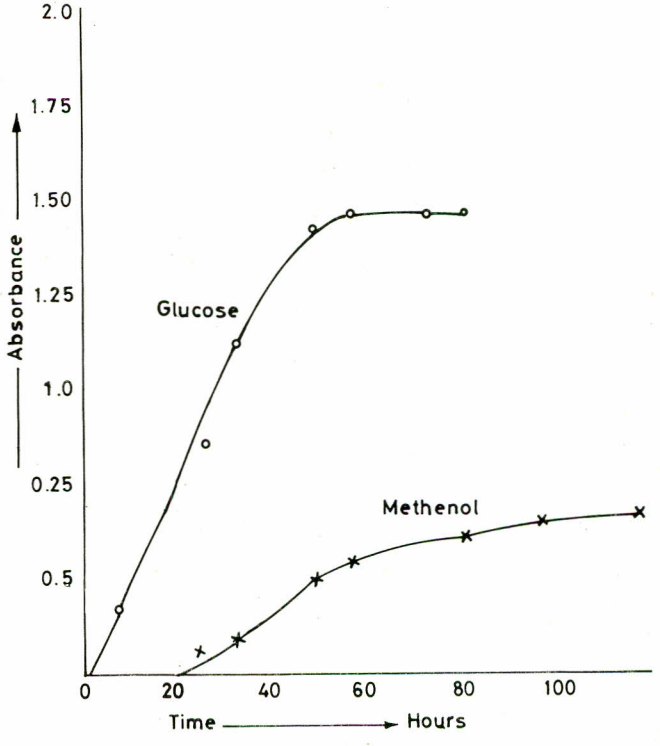

Fig. 1. Growth response of *Candida boidinii* on glucose and methanol.

The difference in 18:2 acid between two carbon sources was different with different concentrations of ammonium sulphate. By varying the ammonium sulphate concentration, variation of fatty acid components took place as shown by GLC analysis. From the figures in the Table 3 it can be inferred that the difference in the fatty acid components noted are significant as the results are reproduceable.

Through the yeast grown on methanol showed about 50 % capacity compared to glucose to form lipids yet the lipid does not appear to contain any components that might be considered undesirable if used as a dietary source.

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