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EFFECTS OF NITROGEN STARVATION ON THE LIPID SYNTHESIS OF *SACCHAROMYCES CEREVISIAE*

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Cultivation of *Saccharomyces cerevisiae* for lipid accumulation has been studied both in the presence as well as the absence of nitrogen in the medium. Under nitrogen starved conditions it has been observed that lipid production increases by hundred percent and the triglycerides decrease by about one hundred and two percent. The overall fatty acid composition of the lipids as studied by Gas Liquid Chromatography shows that palmitoleic (39 %) and oleic acid (45 %) are the major constituents which vary slightly under nitrogen starvation, (Palmitoleic 36 % and oleic 41.7 %).

Key words: Yeast; nitrogen; lipid.

INTRODUCTION

Yeasts have been cultivated as source of fats, and sterols. In brewer's and baker's yeast this amounts to about 4 percent of the dry weight [1]. Certain species of yeasts are however noted for their ability to synthesise appreciable quantities of fat, and such yeasts have been studied as potential source of industrial fats. Reports of upto 23 % lipids in some strains of *Saccharomyces cerevisiae* have also appeared [2]. However, these lipids which appear to accumulate mainly but not exclusively, in the form of lipid granules similar to those seen in oleaginous yeasts, contained substantial proportions of sterols [3]. Sterols upto 12 % of the dry weight of *Saccharomyces fermentati* and upto 10.6 % in certain strains of *S.cerevisiae* have been reported [4]. And in those cases where high levels of total lipids have been reported, almost half of the total turns out to be sterol, either free or as sterol esters. In other strains of *S.cerevisiae,* where the lipid content is likely to be between 8 % to 14 % of the biomass [1] triacyl glycerols do not constitute major lipid fraction and may only represent about 15 % of the total lipids [5].

The number of oleaginous yeasts is comparatively small in relation to the total number of species. However, there are several early reports of fat accumulation in yeasts but these do not seem to have been followed up. It is known that fat accumulation by such strains is affected because of the incubation conditions. Thus fat accumulation in *Saccharomyces cerevisiae* was reported to occur if pregrown cells were incubated with ethanol [6].

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The effects of several hypocholesteremic drugs on lipid metabolism in the yeast have also been examined [7]. Growing *Saccharomyces* in medium deficient in inositol has been found to lead to considerable increases in the lipid content of the cells although the cell yield is halved under the same conditions [8].

Deprivation or addition of vitamins in the same medium have been found to produce changes in the lipid accumulation patterns. The absence of thiamin in the medium is known to give decreased amounts of lipids in *Saccharomyces carlsbergensis* [9]. This aspect, however, will be discussed in more details in a subsequent publication.

In some earlier studies it had been observed that higher lipid percentage is produced when nitrogen percentage is maintained at a bare minimum to those of carbon [10]. It was, therefore, thought interesting to study if complete absence of nitrogen can augment further positive results in enhancing the lipid synthesis. In the present report, therefore, details are given of a study in which *S.cerevisiae* was cultivated exclusively in the absence of nitrogen. It has been observed that nitrogen starvation yielded 100 % increase in the lipid production by *S.cerevisiae.*

MATERIALS AND METHODS

Active dry yeast (*Saccharomyces cerevisiae*) of RYL (Ravi Yeast Limited) was grown aerobically in medium with the following composition.

$K_2H\ PO_4$ = 1.1 %, $KH_2\ PO_4$ = 1.85 % yeast extract 0.2 % NH_4Cl = 0.1 % and glucose = 4.1 %, pH of the medium 5.5, temperature = 28°. The yeast cells obtained

were carefully separated from the nutrient medium by centrifuging. The cells were washed with distilled water and then dried to a constant weight. The percentage of the lipids extracted from these cells has been calculated on the dry wt. basis.

Nitrogen starvation. For nitrogen starvation studies, the mineral composition of the medium was the same as mentioned above but without ammonium chloride. In another experiment the cells to be used as the inoculum were washed with phosphate buffer (pH (5.5) 0.1 molar). These cells were then suspended in the medium which was free from ammonium chloride as well as yeast extract. All other incubation conditions were the same as for the control medium. The cells so obtained were further treated as stated above.

Lipid extraction. The dried cells were grounded with sand in a mortar and lipids were extracted thrice with solvent mixture of methanol, chloroform (1:2 v/v). The extracts were separately filtered and then pooled. The solvent was removed and the residue dried over anhydrous calcium chloride in a vacuum dessicator. The pure lipids were obtained by the subsequent extraction of the dried residue with chloroform.

RESULTS

Effects of nitrogen starvation on lipid percentage in S. cerevisiae. The lipid percentages obtained from the *Saccharomyces cerevisae* cells grown in the presence of ammonium chloride and yeast extract as well as in the absence of these nitrogen resources are given in Table 1. It is seen from the results that the lipid percentage increased from 3.5 % to 7.9 % in the absence of nitrogen source. This increase which is over 100 % indicates that nitrogen starvation phenomenally increases the lipid formation in *S.cerevisiae.*

Table 2 shows that the synthesis of lipids by the yeast cells under nitrogen starved conditions reaches to a maximum value of 7.9 % within 2 hours of the incubation period. Beyond this period significant decrease has been noted.

Fatty acid composition of the lipids. The lipids so obtained under both the conditions were separately evaluated for fatty acid composition.

The extracted lipids were saponified with 0.5N alcoholic potassium hydroxide in the usual manner. The soap solutions were freed from the nonsaponifiable fraction and then acidified with 4N sulphuric acid. After extraction with ethyl ether and removal of the solvent, the liberated fatty acids were converted to their methyl esters [11]. The identity and percentage of the fatty acids was determined by the gas chromatographic analysis (Table 3). The percentage of the triglyceride was also calculated in the extracted lipids. It was found that the triglyceride percentage in the total lipids in the cells grown in the normal and the nitrogen starved media respectively was 35.2 % and 15.0 % whereas total fat increased over 100 %. Triglycerides % in the nitrogen starved cells decreased indicating that lipids other than triglycerides were produced under these conditions.

DISCUSSION

The results of the present study indicate that the nitrogen starvation is favourable for the total lipid synthesis in *S.cerevisiae.* This is so because the absence of nitrogen in the nutrient medium changes the direction of the normal biosynthetic process. Consequently, therefore, the intensity of lipid synthesis is increased proportionately. According to a hypothesis, as a culture becomes

Table 1. Percentage of the lipids showing the effect of nitrogen starvation.

No.	Medium	% of total Lipids
1.	NH_4Cl + yeast extract	3.5 %
2.	Without NH_4Cl but with yeast extract	5.2 %
3.	Without both NH_4Cl and yeast extract	7.9 %

Table 2. Accumulation of lipids with time.

Time	Lipids	
	Without NH_4Cl but with yeast extract	Without NH_4 Cl and yeast extract
2 hours	3.8 %	– 7.9 %
4 hours	4.0 %	6.1 %
6 hours	5.2 %	5.1 %

Table 3. Lipid composition.

	$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
With nitrogen source	–	3.06 %	0.55 %	7.24 %	39.0 %	5.0 %	44.8 %	0.28 %
Without nitrogen source	5.0 %	2.5 %	1.78 %	9.2 %	36.1 %	4.5 %	40.7 %	–

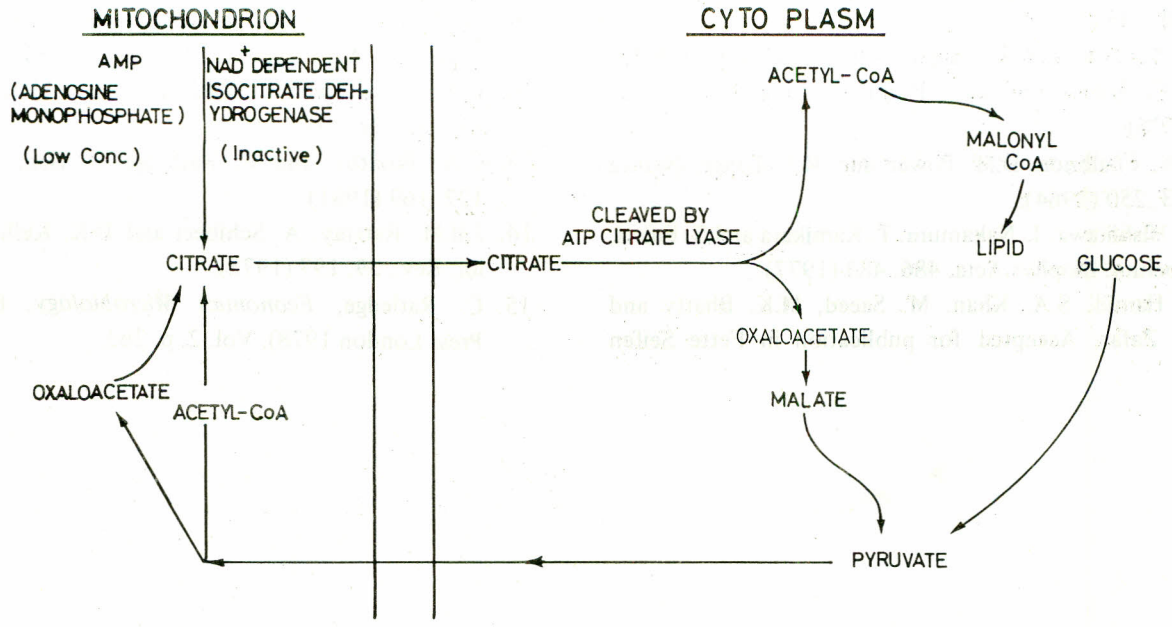

Fig. 1. Pathway of lipid synthesis (with nitrogen starvation).

depleted of nitrogen, the intracellular concentration of AMP (Adenosine mono phosphate) decreases and the mitochondrial NAD^+ dependent isocitrate dehydrogenase, which was a requirement for AMP, becomes inoperative. As a result the citrate accumulates and is cleaved by the enzyme ATP- citrate lyase to produce acetyl -CoA and oxaloacetate. The possession of ATP: citrate-lyase by yeasts thus indicates the potential for oleaginicity [12, 3]. A diagramatic sketch of this pathway is given in Fig. 1. Under such conditions as the cultivation proceeds the oil globule in the cell is markedly developed and finally the cell is almost filled by it has already been noted that several species of yeasts and a large number of moulds can accumulate upto 70 % of their biomass as lipids, when grown with an excess of carbon and deficit of nitrogen [14,15].

In the present studies *S. cerevisiae* has shown over 100 % improvement in lipid accumulation (from 3.5 % - 7.9 %) when grown under nitrogen starved conditions.

Though the lipid accumulation results from the concerted action of several enzymes which appear to show favourable effect under nitrogen starved conditions yet considerable problems arise if lipid accumulation is wanted to be induced into a non-oleaginous organism as the essential metabolic machinery is not there.

Table 3 shows the fatty acid composition of the total lipids in the control as well as the nitrogen starved culture. The major components of the lipids are palmitoleic and oleic acids with a slight decrease in their percentage when grown under nitrogen starved conditions.

It has been observed that *S.cerevisiae*, when grown under normal conditions, produces lipids in which triglycerides amount to 35.2 %. However, under nitrogen starvation conditions this percentage of triglycerides in lipids is decreased to 15.0 %. The occurance of higher quantity of triglycerides in the lipids of oleaginous organisms such as *Lipomyces starkeyi* showing 88 % triglycerides presents a contrast to the lower quantity (35.2 %) of triglycerides in the lipids of non oleaginous strain such as *S.cerevisiae.* However, this percentage of triglycerides in the lipids of *S.cerevisiae* compare favourably with the already reported percentage.

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