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TEMPERATURE EFFECTS IN THE BIOSYNTHESIS OF FATS AND FATTY ACIDS OF *CANDIDA UTILIS*

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The influence of temperature on the growth, lipid production and fatty acid composition of *Candida utilis* is discussed. A decrease in growth temperature from 30-15° has been shown to result in increased lipid formation and higher ratio of linoleic acid to oleic acid. An increase in lipid unsaturation at low temperature and its decrease at high temperature has been observed as the dominant response.

Key words: Biosynthesis, *Candida utilis*, Unsaturation, Glycolipids, Phospholipids.

Introduction

Temperature, directly or indirectly, is one determinant in altering biosynthesis and distribution of fats and fatty acids. The gel/liquid crystaline transformations are an integral part of the phase changes by which naturally occuring lipids may respond to an environmental stress such as temperature. Both in nature and laboratory, an increase or decrease in temperature may cause lipids respectively to liquify or to solidify.

Micro-organisms have been the primary experimental material for studying the effects of temperature on the chemistry of fatty acids. It has been observed in organisms ranging from bacteria, protozoa, fungi and algae to higher plants and animals that the proportion of unsaturated to saturated fatty acids in a cell or tissue increases within limits with decreasing environmental temperature [1].

One of the biochemical mechanisms in times of thermal stress is an adjustment of lipid unsaturation; low temperature survival favours an increase in lipid unsaturation whereas high temperature leads to a decrease in lipid unsaturation. The micro-organisms thus have several strategies to produce fatty acids with lower melting points in response to decreasing temperature. These include for example, changes in chain length of fatty acids, levels of fatty acids branching, hydroxylation, cyclization and the distribution and relative pro-portions of members of the glycolipid and phospholipid families [2].

In common with most living organisms, yeasts generally show a tendency to raise the lipid content and degree of unsaturation as the environmental temperature is dropped below that for optimum growth. Temperature effects on growth, lipid production and fatty acid composition of *Candida utilis* forms the basis of the present study. It was essentially undertaken to gather more knowledge about the idea that lipids of living systems can respond in several ways to temperature stress yielding a variety of fatty acids.

Experimental

Candida utilis was maintained on slopes of malt extract/yeast extract/glucose/peptone/agar. It was further grown on medium containing KH_2PO_4: 7.0; Na_2HPO_4, 2.0, $MgSO_47H_2O$, 1.5; yeast extract, 1.5; $CaCl_26H_2O$,2; $FeCl_36H_2O$, 0.1; biotin, 0.001; and $ZnSO_4$ $7H_20$, 0.001 gm/litre. The pH was adjusted to 5.5 with HCl. Glucose and NH_4Cl were supplied for limited growth at 30 and 1.5gm/litre respectively. Organisms were harvested by filtration. Batches of harvested organisms were washed twice with ice cold distilled water, removed from the filter by shaking in ice cold water and the suspension was decanted and freeze dried.

Determination of yeast dry weight. Two samples (10 ml) were centrifuged at 5000 gm for 10 mins washed twice with distilled water (10 ml) and dried at 100° (24 hrs) to constant weight.

Lipid extraction. The frozen cells were transferred quantitatively into two volumes of ethanol and diethyl ether (3:1). After decanting the solvent, the cells were extracted twice for 2 hrs using methanol, chloroform solvent system (2:1). The combined solvent mixture was filtered through Whatman No. 1 filter paper and washed twice with diethyl ether. The solvent was evaporated at 50° under vacuum [3].

To the residual material ION methanolic KOH was added and lipids were saponified under relux for 30 mins and acidified to pH2. The liberated fatty acids were extracted and methylated by refluxing for 30 mins in 5% methanolic HCl. The methyl esters were concentrated by pouring the reflux mixture into two volumes of cold saturated sodium chloride solution. After three washings the methyl esters were separated and stored in deep freezer [4].

Results and Discussion

Contents of neutral lipid triglycerides and esterified sterols and free sterols showed no significant differences throughout the cell cycle. However, the phospholipid content varies throughout the cycle, and these contents in organisms

harvested after 24 and 32 hrs were significantly different from those in organisms harvested at other times (48, 56 and 72 hrs). The major effect of low temperature on phospholipid fatty acids of *C. utilis* was an increase in linoleic acid mainly at the expense of lenoleic acid (Table 1).

Effect of glucose concentration on lipid formation. The total lipid extract from yeast cells was shown to increase with increasing glucose concentration and the dry cell weight increased almost linearly until nitrogen in the broth was exhausted (Table 2). Limitation of growth by the supply of carbon occurred when glucose was 4% and NH_4 Cl was 3.0gm/l. Under these conditions the minimum lipid content of the yeast was 9% wt/wt and the maximum was 14% wt/wt (Table 3). At this stage the lipid production was stimulated and the biomass weight remained constant. The lipid content was maximum during the stationary phase and rapid decline occurred with the exhaustion of glucose in culture.

Effect of temperature on lipid formation and fatty acids. The fatty acids composition of the cells varies with growth temperature, fermentation time and medium. Fatty acids produced at 15° were slightly more unsaturated than at the optimum temperature.

The growth of *C. utilis* results in higher proportion of $C_{18:1}$ and $C_{18:3}$ at 15° compared to 30° (Table 4). The specific alterations in $C_{16:0}$ $C_{18:1}$ and $C_{18:2}$ acids are subject to limitation of glucose or nitrogen in the medium. (All experiments, described here, were performed more than once).

Table 4 illustrates that decrease in temperature increased the amounts of linoleic and linolenic acids at the expense of less unsaturated fatty acids particularly oleic acid. This general trend of increased unsaturation in response to decreasing temperature, however, is dependent on species variation. For example in the fungus *Neurospora erassa* the major low temperature effect in phospholipid fatty acids is an increase in linolenic acid at the expense of linoleic acid [5]. In variation to this in the total lipids of the yeast *Hansenula polymarpha* linolenic acid increases at the cost of a wide variety of less unsaturated fatty acids [6]. A more dramatic species difference, however, is shown with the bacterium *pseudomonas aeroginosa*. Here a decrease in temperature increases not only the more unsaturated palmitoleic and oleic acids at the expense of stearic, palmitic and laurie acids but also result in an increase in the hydroxylated fatty acids of the total lipids [7].

Because of these variations in the effects of low temperature on the fatty acid composition of a diverse variety of microorganisms' various regulatory mechanisms have been proposed. For eucaryotic cells these are modulation of the existing desaturase enzyme molecules by membrane fluidity, synthesis of new desaturase enzyme molecules induced by low temperature and desaturase inhibition by polyenoic fatty acids [8]. For procaryotic cells there are offered other regulatory mechanisms. In *Escherichia coli* a temperature labile enzyme (ketoacyl - ACP synthase) specifically increases the rate of unsaturated fatty acid synthesis at low temperature [9]. In *B. megaterium* the desaturase induction is mediated by a temperature sensitive modulator, temperature labile at 35° and most active therefore, at low temperature [10].

In addition, the effect of temperature, may be mediated through or modulated by a number of other factors that are part of the fermentation process, be it batch or continuous. These include oxygen availability, nature of nutrients, dilution rate and growth stage. Two other aspects of the experimental

TABLE 1. EFFECT OF TEMPERATURE CHANGE IN FATTY ACIDS OF PHOSPHOLIPIDS OF *CANDIDA UTILIS*.

Fatty acid	Relative %		Difference
	15°	30°	30-15°
$C_{16:0}$	12.0	18.8	-6.7
$C_{18:1}$	5.2	9.3	-4.1
$C_{18:2}$	28.4	59.7	-21.3
$C_{18:3}$	44.3	12.2	+32.1

TABLE 2. GLUCOSE AND NITROGEN UTILIZATION DURING CULTIVATION OF *CANDIDA UTILIS*.

Time period in hrs.	Sugar %	NH_4Cl%
Initia 1	4.0	3.0
After 8	3.5	2.5
" 24	2.9	2.0
" 32	2.0	2.0
" 48	1.0	1.2
" 56	0.5	0.6
" 72	0.2	0.3

TABLE 3. EFFECT OF DIFFERENT SUGAR CONCENTRATIONS ON THE LIPIDS FORMATION OF *CANDIDA UTILIS*.

Sugar concentration	Biomass gm/l	Lipid % on dry wt. basis
2.0	4.5	9.0
3.0	5.5	12.0
4.0	6.0	14.0
5.0	6.0	14.0
10.0	6.0	14.0

TABLE 4. EFFECTS OF TEMPERATURE CHANGE IN FATTY ACID COMPOSITION OF *CANDIDA UTILIS*.

Fatty acid	Relative %		Difference
	15°	30°	30-15°
$C_{16:0}$	13.2	24.0	–10.8
$C_{16:1}$	11.5	11.0	+0.5
$C_{18:0}$	4.3	6.5	–2.2
$C_{18:1}$	10.7	26.5	–15.8
$C_{18:2}$	29.7	19.1	+10.6
$C_{18:3}$	32.5	10.6	+21.9

protocol also can be involved, i.e., species to species variabi-lity in response, as described above, and the lipid material under investigations whether it be total lipids or a particular class such as phospholipids, glycolipids and triglycerides.

However, given that exceptions and alternatives exist it is still reasonable to expect that reducing temperature will increase lipid unsaturation. To achieve this objective, however, the biosynthetic steps should be performed at the lowest temperature compatible with the demands of technology and economics. The influence of other variables that may modulate the inverse relationship between temperature and unsaturation should also not be neglected.

The present study, therefore, strengthens the idea that the lipids of living systems can respond in several ways to temperature stress, yielding a variety of fatty acids. These findings may help to synthesize fats and fatty acids of modified structures and proportions from microorganisms in response to thermal states. It affords a survival advantage to living system in many instances such as the production of indus-trially important polyunsaturated fatty acids, unsaturated wax esters and unsaturated edible oils.

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