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FEATURES OF SYNTHESIS OF FATTY ACIDS IN ACTIVE LIPID-FORMING YEASTS

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Features of the accumulation of fatty acids and the changes in their composition in seven strains of yeasts that are promising producing agents of lipids have been studied. It has been shown that in the process of accumulation of lipids in the biomass changes take place that lead to a fall in the degree of unsaturation of the pool of fatty acids. This nature of the change in the composition of the fatty acids is, as has been shown by measuring their absolute amounts in the biomass, connected with the intensive synthesis of oleic and palmitic acids as components of the reserve lipids. The results obtained form the basis for an evaluation of the promising nature of individual strains as active producers of fatty acids.

The chemical and physical properties of natural lipids that are responsible for their functional significance and also for the possibility of their use in various sectors of industry are determined primarily by their fatty acid composition.

In recent years, in our country and abroad, active investigations directed discovering substitutes for food oils and fats used for technical purposes have been carried on. Fats of microbial origin, and, especially, yeast lipids, are considered as the most promising from this point of view. The prerequisites for such substitution are a predominance of triacylglycerols in the fractional composition of yeast lipids and the presence among their fatty acids of appreciable amounts of unsaturated acids [1, 2] and, in particular, of linolenic acid [3].

In view of this, in the choice of lipid-producing microorganisms a knowledge of the fatty acid composition of their lipids and the limits of its change with the aid of various technological factors without intervention in the hereditary apparatus of the organism acquires prime importance.

In the present paper we consider the laws of the synthesis of fatty acids by "fatty" strains of yeasts under the conditions of the active synthesis of lipids. Seven strains of yeast were investigated: Candida beechii VKM u-1428, Lipomyces lipofer B-5, Rhodotorula glutinis var. glutinis DKM u-329, 334, 337, 1630, and Rh. gracilis BKM u-335 (all obtained from the All-Union Collection of Nonpathogenic Microorganisms at the Institute of the Biology and Physiology of Microorganisms of the USSR Academy of Sciences) [4], which are capable of accumulating up to 20-50% of lipids [5].

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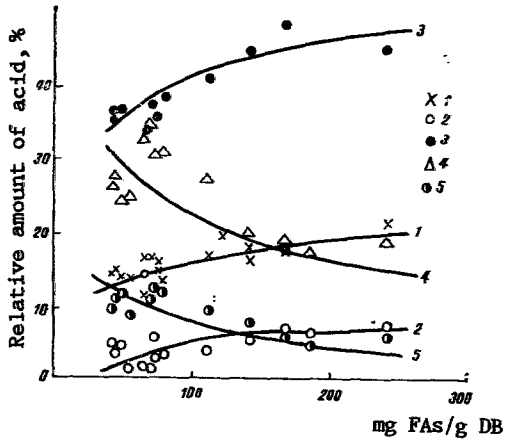

Fig. 1. Dependence of the relative amount of the main fatty acids (FAs) in the dry biomass of the strain Rh. gracilis VKM u-335 on the level of accumulation of lipids in it: 1) 16:0; 2) 18:0; 3) 18:1; 4) 18:2; 5) 18:3.

A study of the dynamics of the fatty acid composition of the total lipids of the selected "lipid" strains of yeast showed that, regardless of their genus and species affinity, an increase in the amount of lipids in the biomass was accompanied by the same changes in the composition of the fatty acids. They were expressed in a fall in the relative amount of polyunsaturated C_{18} acids – linoleic and α-linolenic acids (18:2 and 18:3) – with a simultaneous increase in the proportion of palmitic (16:0), stearic (18:0), and oleic (18:1) acids. The most considerable change in the composition of the fatty acids took place on an increase in the amount of total lipids in the biomass from 40-60 to 150-250 mg of FAs/g of dry biomass (DB). At higher concentration of lipids the relative composition of fatty acids changed very little, as is shown in Fig. 1 for the strain Rh. gracilis VKM u-335.

With an increase in the amount of lipid in the biomass of the culture from 68.8 to 282.9 mg FAs/g DB, the relative amounts of the 16:0, 18:0, and 18:1 acids rose from 16.3 to 25.3%, from 4.8 to 12.1%, and from 31.9 to 41.8%, respectively. At the same time, the proportion of the 18:2 and 18:3 acids fell from 36.3 to 15.4% and from 7.7 to 3.8%. The process of subsequent lipid formation took place with no change in the fatty acid composition and, consequently, with no increase in the unsaturation of the lipids synthesized. A similar pattern was observed for the other strains investigated.

This nature of the change in the fatty acid composition of the lipids of active lipid-forming yeast cultures permits, as we assume, an explanation of the contradiction in experimental results in papers on the study of the influence of the rate and time and growth of yeast cultures on the chemical composition of the total lipids. In this case, when cultivation is carried out under conditions ensuring a gradual increase in the concentration of lipids in the biomass, a considerable dependence of the fatty acid composition of the lipids synthesized on the factor under investigation is observed. Such results have been obtained, for example, for cultures of Rh. glutinis 35 [6, 7] and Lipomyces starkeyi [8]. In those cases where the level of accumulation of lipids in the biomass over the whole range of investigations was high (more than 20-30% of the DB), the composition of the fatty acid scarcely changed, as was shown in the periodic cultivation of the yeast Trichosporon pullulans and Cryptococcus albidus var. aerius [9].

In a study of the process of formation of lipids by the "fatty" cultures under investigation, experimental results were also obtained on the accumulation of the absolute amounts of the fatty acids of the yeast lipids with a rise in the fat content of the biomass. The experimental results were treated on a Nairi-K computer by a program of "correlation-regression analysis of two random magnitudes," and equations were obtained that described the process for the synthesis of each acid (mg per 1 g of DB) as a function of the total amount of fatty acids in the biomass (mg per 1 g of DB) (Fig. 2).

It has been shown that the dependences are, in the main, linear and have a rising nature, in contrast to the dynamics of the individual amounts of fatty acids. For all the strains considered, without exception, within the limits of accumulation of lipids investigated (averaging from 40 to 150-400 mg FA/g DB), the 18:1 acid predominated, its amount in individual cases reaching 200-250 mg/g DB. The other acids of the strains L. lipofer B-5, Rh. glutinis var. glutinis VKM u-329, 334, and 1630, and Rh. gracilis VKM u-335 formed the following sequence with respect to the level of accumulation in the biomass: 16:0 > 18:2 > 18:0 > 18:3 (Fig. 2a). For the strains C. beechii VKM u-1428 and Rh. glutinis var. glutinis VKM u-337, the 18:1 acid was followed by the 18:2 acid, and the amount of the 18:3 acid exceeded the amount of the 18:0 acid (Fig. 2b).

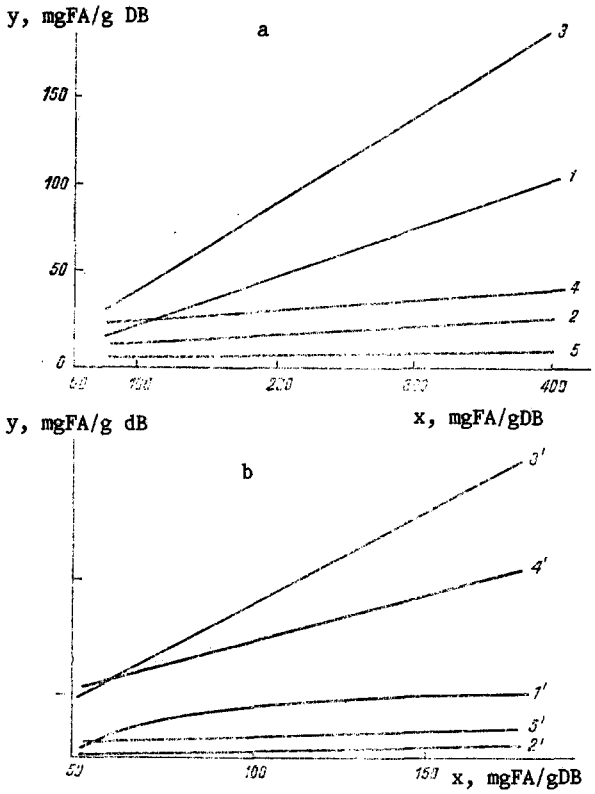

Fig. 2. Dynamics of the accumulation of the main fatty acids by the strains Rh. gracilis VKM u-335 (a) and C. beechii VKM u-1428 (b):

1 — 16 : 0 — $y = a + bx$,	$a = -10.142$, $b = 0.293$;
2 — 18 : 0 — $y = a + bx$,	$a = 4{,}196$, $b = 0.068$;
3 — 18 : 1 — $y = a + bx$,	$a = -4.618$, b = 0.457;
4 — 18 : 2 — $y = a + bx$,	$a = 16.516$, $b = 0.077$;
5 — 18 : 3 — $y = a + bx$,	$a = 3{,}422$, $b = 0.025$;
$1'$ — 16 : 0 — $y = a + bx$,	$a = 22.256$, $b = -965.901$;
$2'$ — 18 : 0 — $y = a + bx$,	$a = -0.895$, $b = 0.025$;
$3'$ — 18 : 1 — $y = a + bx$,	$a = -6{,}672$, $b = 0.505$;
$4'$ — 18 : 2 — $y = a + bx$,	$a = 7{,}620$, $b = 0.255$;
$5'$ — 18 : 3 — $y = a + bx$,	$a = 2{,}595$, $b = 0.041$,

where x is the total amount of fatty acids in the biomass, mg FA/g DB; y is the amount of an individual acid in the biomass mg FA/g DB; and a and b are coefficients of the equation.

Thus, in the processes of accumulation of the main fatty acids, apart from the 18:1 acid, we did not succeed in finding either general-genus or general-species features. The coefficients of proportionality of the relationships established for the accumulation of fatty acids were different. Thus, the angular coefficients had the greatest value for the 18:1 and 16:0 acids, the rapid and considerable accumulation of which in the biomass probably reflects a process of intensive synthesis of reserve triacylglycerols, the main acids of which they are [10]. At the same time, the small value of the angular coefficient for the 18:3 acid, which is usually concentrated in the structural lipids of the yeast cells [10], permits the assumption that their absolute amount in the biomass scarcely changes on the passage of the culture from one stage of development to another.

The results obtained also permit the potential possibilities of each strain as a producer of the 18:2 acid, the only essential fatty acid synthesized by yeast [3, 6], to be determined. Thus, for example, of all the cultures investigated, the synthesis of the 18:2 acid took place most intensively for the strain C. beechii VKM u-1428: at a concentration of total lipids in the biomass of only 20% on the DB (which corresponds to 150 mg FAs/g DB) the amount of the 18:2 acid was 50 mg/g DB (Fig. 2b).

EXPERIMENTAL

The cultures were grown on a circular shaking machine (200-220 rpm) at a temperature of 26-28°C in conical flasks (V = 750 ml) containing 100 ml of nutrient medium of the follow-

ing composition (g/liter): $NH_4H_2PO_4$ – 4.0; Na_2HPO_4 – 7.0; KH_2PO_4 – 6.8; $MgSO_4 \cdot 7H_2O$ – 0.7; yeast autolysate – 5.0. The source of food and energy carbon was ethanol, which was added to the medium in an amount of 2% by volume. The initial pH of the medium was 6.3-6.5.

The lipids of the yeast cultures were analyzed by methods permitting the determination not only of the composition of the fatty acid spectrum but also the absolute amount of each acid in the biomass [11]. The total amount of fatty acids in milligrams per 1 g of DB was used as an index of the level of accumulation of the total acyl-containing lipids in the yeasts.

CONCLUSIONS

1. Using, as examples, seven "fatty" cultures of the genera Candida, Lipomyces, and Rhodotorula, it has been shown that the process of lipid formation in the yeast cell is accompanied by a fall in the degree of unsaturation of the lipids synthesized through an increase in the proportion of palmitic, stearic, and oleic acids and a decrease in the concentration of linoleic and α-linolenic acids.

2. On the basis of a correlation-regression analysis of the statistical results obtained, a linear rising nature of the dependence of the absolute amounts of the main fatty acids of the yeast lipids on the fat content of the biomass has been established. A comparison of the proportionality coefficients of the relationships permits the assumption of a predominance of the synthesis of reserve lipids in the yeast cells above the synthesis of structural lipids, the amount of which scarcely changes.

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