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Biosynthesis of Lipoxygenase, Lipids and its Fatty Acid Composition of Actinomycetes and Yeast

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Abstract

Biosynthesis of lipoxygenase, lipids and the fatty acids composition of Streptomyces canosus 71, S. massasporeus 36 and Candida utilis were investigated under using various media. Existence of the correlation between lipid biosynthesis, its unsaturation degrees on the one hand and lipoxygenase activity on the other hand were established. The cultures with low lipid content and high level of unsaturated fatty acids, as a rule, possessed the most enzyme activity.

Keywords: *Streptomyces canosus 71, S. massasporeus, Candida utilis,* lipids, lipoxygenase, fatty acids.

Introduction

The interest to investigation of lipid metabolism enzymes by various microorganisms is bound with opening the varied lipid role in the physiology of intracellular processes and with the possibility of regulating its composition.

Much attention is directed to lipoxygenase synthesized microorganisms; it connects with the great practical importance of the enzyme (1, 2).

Literature data about lipoxygenase synthesis are extremely limited; it causes definite difficulties during investigation.

Lipoxygenase catalyses the oxidation of polyunsaturated fatty acids, takes part in the prostaglandin synthesis by action on the arachidonic acid – predecessor of these compounds. The enzyme role in metabolic turning of fatty acids discovers the great possibility of receiving of natural compounds with the great biorelated properties (1, 3).

This work presents the results of the lipoxygenase biosynthesis investigations and its possible correlation with the lipogenase activity and fatty acid composition of lipids, synthesized by *Streptomyces canosus 71, S. massasporeus 36* and *Candida utilis* under usage of various media.

Materials and Methods

Microorganisms were grown in the condition of submerged cultivation in 750 ml flasks with 200 ml of following media (%): the soybean medium as describe earlier (1), M-1: maize flour – 2, dry yeast – 0,15, $CaCO_3$ – 0,15 , pH – 7,0 ; Lundin's medium: $(NH_4)_2SO_4$ – 0,1, K_2HPO_4 – 0,1 , NaCl –0,1 , $MgSO_4$ – 0,1 , $FeCl_3$ – 0,01 , sugar – 5,0.

Separation of lipids was carried out by usual methods (4) with modified procedures of cell wall destruction and extract agents utilization.

Fatty acids were determined by gas-liquid chromatography (5).

Cells were destroyed using ultrasound in ice cold conditions (1).

The homogenate was centrifuged at 18000 g at 4^{o} and the resulting supernatant was carefully collected and immediately used for the assay.

Lipoxygenase activity was determined using polygraph method with Clark electrode (1).

Linolenic acid was used as substrate. Unit of the enzyme activity is defined as the joining amount of nmol oxygen to the substrate per 1 sec under action of 1 mg of enzyme.

Results and Discussions

As shown in the **Table 1**, the maximum amount of biomass both in actinomycetes and in the yeast was noted under culturing for 5 days. Medium M-1 was more favorable to lipid synthesis of actinomycetes.

Table 1. Biomass and lipid content of actinomycetes and yeast cultured for 3 or 5 days.

Cultures	Culture media	Biomass, $^{g}/_{l}$		Lipids, % of dry biomass	
		3 days	5 days	3 days	5 days
		M $^{+}$/-m	M^{+}/-m	M^{+}/-m	M^{+}/-m
Streptomyces canosus 71	Soybeans	1,7+/-0,1	2,11+/-0,11	2,63+/-0,18	6,6+/-0,36
	M – 1	5,58+/-0,25	9,2+/-0,75	9,4+/-0,32	10,96+/-0,17
Streptomyces massasporeus 36	Soybeans	3,35+/-0,13	7,37+/-0,41	5,13+/-0,09	8,33+/-0,43
	M – 1	7,21+/-0,27	10,67+/-0,87	7,16+/-0,14	11,5+/-0,38
Candida utilis	Soybeans	3,16+/-0,14	4,13+/-0,13	11,47+/-0,26	12,07+/-0,43
	Lundin's	1,87+/-0,69	2,91+/-0,26	5,2+/-0,17	5,98+/-0,07

Biomass and lipid biosynthesis both of *S. massasporeus 36* and *S. canosus 71* didn't differ considerably. Soybean medium was more favorable for yeast than the Lundin medium. As a result the amount of biomass and lipids on the average was twofold higher.

The highest level of lipoxygenase activity both of *S. canosus 71* and of *C. utilis* was established under cultivating for 3 days (2.25 $^{u}/_{mg}$ and 2.15$^{u}/_{mg}$ respectively) (**Table 2**). The culture *S. canosus 71* was more active than *S. massasporeus 36*. As a rule, lipoxygenase activity was higher in the species, cultured for 3 days. Activity of the most active from all investigated species *C. canosus 71*, cultured for 3 days, exceeded up to ten times as compared to one, cultured for 5 days, (2.25 $^{u}/_{mg}$ and 0.238 $^{u}/_{mg}$ protein respectively). Culture *S. massasporeus 36* was an exception: lipoxygenase activity was higher, when cultured for 5 days.

Table 2. Lipoxygenase activity of actinomycetes and yeast cultured for 3 or 5 days.

Cultures	Culture media	Lipoxygenase activity (u/mg protein)	
		3 days	5 days
Streptomyces canosus 71	Soybeans	2,25+/-0,01	0,24+/-0,0
	M – 1	1,17+/-0,01	0,23+/-0,017
Streptomyces massasporeus 36	Soybeans	0,29+/-0,01	1,0+/-0,09
	M – 1	0,35+/-0,006	0,181+/-0,001
Candida utilis	Soybeans	0,42+/-0,02	0,44+/-0,023
	Lundin's	2,15+/-0,05	1,17+/-0,0174

It was interesting to note that the soybean medium was the most favorable to lipoxygenase activity of actinomycetes than M-1. On the other hand, the enzyme activity of yeast was higher under cultivating on Lundin's medium than on the soybean medium (in 2-5 times).

As shown in this experiment, it was necessary to note the existence of correlation between lipogenase and lipoxygenase activities (**Table 1, 2**). So, all investigating species, cultured for 5 days had the highest level of lipogenase activity than species, cultured for 3 days. The opposite picture revealed under the analysis of lipoxygenase activity. As a rule, its maximum was noted at 3 days of growth.

Under analysis of the used mediums the similar regulate was revealed. Under using M-1 medium the maximum of lipoxygenase activity was noted, when using soybean the minimum was one.

The highest lipogenase activity of yeast *C. utilis* was under use of the soybean medium as compared to using Lundin's (11.47% and 5.2% at 3 days). On the contrary, lipoxygenase activity of yeast was minimum under usage of the soybean medium (0.42 $^{u}/_{mg}$ protein) and maximum (2.15 $^{u}/_{mg}$) – under using Lundin's.

As shown obtained results, the level of biomass accumulation concurred with lipoxygenase activity. As a rule, the maximum of biomass synthesis was fixed in the version, where the minimum of enzyme activity was noted.

It is known that lipoxygenase catalyses the oxidation of certain polyunsaturated fatty acids to form conjugated dine hydroperoxides (3). It has been reported, that the change in the quantity of lipids peroxides, accumulating actinomycetes during cultivation connected with the processes of the increase of biomass by a culture (1). Probably, accumulating peroxides inhibits lipoxygenase activity. That fact can explain, that the enzyme activity decreases during biomass accumulation.

It is well known that lipoxygenase is an enzyme, taking part in the metabolic processes of unsaturated fatty acid composition. It was very interesting to investigate the fatty acid composition in the versions, where it was noted the difference in lipoxygenase activity (**Table 3, 4**).

Table 3. The fatty acid composition of actinomycetes lipids cultured on M-I medium for 3 or 5 days (%).

Fatty acids	*S. canosus 71*		*S. massasporeus 36*	
	3 days	5 days	3 days	5 days
$C_{14:0}$	-	0,59	-	-
$C_{16:0}$	14,53	26,01	15,03	18,21
$C_{16:1}$	5,01	9,43	1,67	3,75
$C_{17:0}$	-	10,4	-	7,5
$C_{17:1}$	-	-	-	1,88
$C_{18:1}$	28,16	52,02	78,91	61,6
$C_{18:2}$	52,3	1,56	4,38	7,07
Sum of unsaturated acids	85,47	63,01	84,96	74,29
Lipoxygenase activities u/mg protein	1,17	0,23	0,35	0,18

It was necessary to note that the contents of unsaturated fatty acids of *S. canosus 71* and *S. massasporeus 36* decreased during growth under usage of the M-1 medium; the level of lipoxygenase activity was lower too (**Table 3**).

Similar data were obtained, using *C. utilis*, growing on different media (**Table 4**). The unsaturated fatty acids content reached 78.69%, lipoxygenase activity – 1.17 $^{u}/_{mg}$ protein under usage Lundin's medium. A decrease in both unsaturated fatty acids (56.45%) and lipoxygenase activity (0.44 $^{u}/_{mg}$ protein) under using soybean medium was noted.

According to the data in the scientific literature, actinomycetes of the *Streptomyces genus* and yeast of the *Candida genus* have high lipoxygenase activity (1).

Our data showed, that by investigating culture *S. canosus 71* and *C. utilis* we have a high ability of lipoxygenase biosynthesis: activity level achieved 2.25 $^{u}/_{mg}$ protein and 2.15 $^{u}/_{mg}$ protein accordingly.

The regulation of lipoxygenase synthesis mediatory connects with quantity and quality content of fatty acids in actinomycetes cell membranes. The maximum of the enzyme formation coincides with maximum of polyunsaturated fatty acids content in lipids (1).

Table 4. Fatty acids composition of lipids *C. utilis* cultured for 5 days (%).

Fatty acids	Lundin medium	Soybean medium
$C_{14:0}$	-	0,12
$C_{15:0}$	-	0,67
$C_{16:0}$	19,2	39,44
$C_{16:1}$	0,64	7,74
$C_{17:0}$	2,12	2,32
$C_{17:1}$	-	0,87
$C_{18:1}$	68,17	44,75
$C_{18:2}$	9,88	4,09
Sum of unsaturated acids	78,69	57,45
Lipoxygenase activity u/mg protein	1,17	0,44

According to literature data, in the young growing culture the role of functionality significant lipids, especially, lipids with considerable quantity of polyunsaturated fatty acids, is very high. The increasing of lipids in ageing culture takes place, obviously with increasing of part of the components, which act as stock energy materials (6, 7). That is explained by the high level of lipoxygenase activity in the young culture, connected with unsaturated fatty acids transformations, their content being higher in this period.

We have shown that lipids content in all the used cultures had been considerably higher on the 5-th day of growth in comparison with 3rd day. The biosynthesis of polyenic fatty acids correlates with lipoxygenase biosynthesis, that takes part in metabolism and co-transformations of these acids (1, 8). It is obvious, that on the 3rd day of growth the level of activity is the highest and on the 5th day it falls considerably. The analogic conclusion is made by other authors, having shown that in that period the polyunsaturated fatty acids content considerably increased. It was determined that in *S. spheroids* the considerable increase of lipoxygenase synthesis level was observed to 70-72 hours of growth, that corresponded to the end of the exponential phase (1). The same observations can give a reason to assume that the essential factors determined the level of lipoxygenase synthesis are either interconnection of this enzyme with the ages state of microbial cell (with the constitutive processes in the cell), or it connects with the changing of the culture medium content, with the presence of the certain compounds (1, 3, 8).

We can assume can suppose that using medium M-1 for actinomycetes, when the general increase of lipids content was noticed, this increase occurs at the expense of the functional components growth. As the result, it can be noticed the decrease of lipoxygenase activity. For yeast *C. utilis* the soybean medium was the most favorable for lipids biosynthesis, but lipoxygenase activity in this medium noticeably lower in comparison with Lundin medium.

Thus, the adduced data testify that the correlation between lipids accumulation in the culture and intracell lipoxygenase activity exists. When the lipid content increased in the ageing culture, it could be noticed the falling of lipoxygenase enzymatic activity. Using the culture media, favorably acting on culture lipogenase activity, negatively influenced on lipoxygenase activity. The highest lipoxygenase activity, as a rule, was noted in the culture with low contents of lipids and biomass.

It was noticed the high level of unsaturated fatty acids content in those variants, where high lipoxygenase activity was registered.

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