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SHORT COMMUNICATIONS

Interrelation of the Biosyntheses of Lipids, Lipoxygenase, and Lipase in Cultured Streptomycetes

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The interest in studies of lipid biosynthesis in microorganisms is frequently dictated by their promise as model objects for clarifying the functional role of lipids in cellular metabolic activity [1]. Such clarification heavily depends on the relation between the biosynthesis of lipids and the biogenesis of the enzymes involved in their production and metabolic transformations. Lipoxygenase (LOG; EC 1.13.11.12) and lipase (triacylglycerol hydrolase; EC 3.1.1.3) provide examples of such enzymes. The data available from previous publications relate to biosyntheses of the enzymes involved in lipid metabolism in microorganisms of diverse taxa [2, 3]. There is little information, however, on the interrelation between the biosynthetic pathways leading to these enzymes, on the one hand, and to intracellular lipids, on the other. Such information may be quite valuable, since cleavage of membrane lipids by lipase supplies substrates with fatty acid radicals for LOG. LOG acts on arachidonic acid; hence its involvement in prostaglandin biosynthesis, which, in turn, opens up the possibility of obtaining highly active bioregulators. Reactions catalyzed by LOG initiate syntheses of compounds affecting the processes of growth, development, and differentiation of organisms. Signal molecules causing cells to alter their metabolism (depending on physiological factors) are also formed [4]. The available evidence has largely been obtained in experiments with plant objects and tissue cultures but not in microorganisms.

In this work, we sought to study the biosyntheses of lipids, LOG, and lipase in streptomycetes cultured on diverse media and at variable stages of growth, with a view to establishing possible interrelations between these biosynthetic processes.

We studied cultures of streptomycetes (*Streptomyces canosus* 71 and *S. massasporeus* 36) from the National Collection of Non-pathogenic Microorganisms of Moldova. The microorganisms were grown in the form of submerged cultures at 28°C and 180–200 rpm in 750-ml flasks, each of which contained 200 ml of soybean medium or M-1 medium. The soybean medium had the following composition (%): glucose, 3; sodium citrate, 0.4; K_2PO_4, 0.2; $Mg_2SO_4 \cdot 7H_2O$, 0.57; $(NH_4)_2SO_4$, 0.125; $ZnSO_4 \cdot 7H_2O$, 0.01–0.02; and soybean extract, 5. The medium M-1 had the following composition (%): corn flour, 2; dry yeasts, 0.15; and $CaCO_3$, 0,15. In both cases, the pH was neutral (7.0).

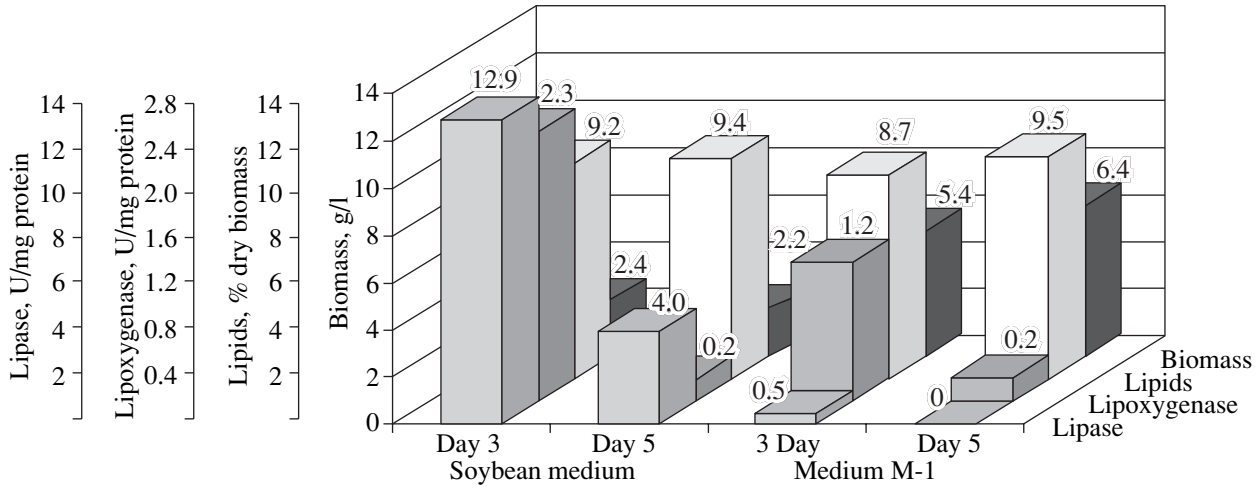

Fig. 1. Biomass accretion, lipid accumulation, and the activity of lipase and lipoxygenase in cultured *S. canosus* 71.

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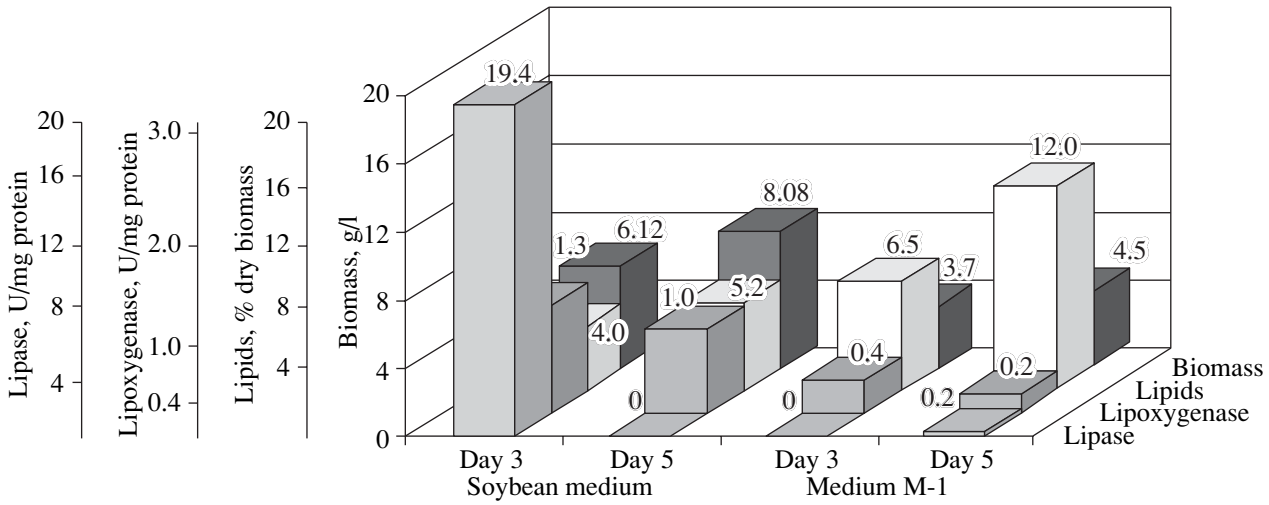

Fig. 2. Biomass accretion, lipid accumulation, and activities of lipase and lipoxygenase in cultured *S. massasporeus* 36.

The lipids were isolated as described by Kates [5]. The activity of the lipases was determined using a modified form of the method of Ota and Yamada [6]. One unit of activity was defined as the amount of enzyme needed to cleave 1 μmol oleic acid per h during hydrolysis of 40% emulsion of soybean oil in 2% polyvinyl alcohol (37°C). The activity of LOG was measured by polarography using a Clarke electrode [7]. The amount of oxygen (nmol) added to linolenic acid per s in the presence of 1 mg of the enzyme was taken to be equal to one activity unit.

Figures 1 and 2 show data on the accumulation of the biomass, lipids, lipase, and LOG in the cultured actinomycetes. The highest LOG level was detected in the culture of *S. canosus* 71 on day 3 of growth. The soybean medium provided a more favorable environment for the biosynthesis of this enzyme. The activity of LOG attenuated as the cultures grew older.

The highest lipase activity was detected in *S. massasporeus* 36, which was grown in the soybean medium but supplemented with an inducer (the value measured under these conditions was more than 10 times higher than that detected in the medium M-1). Maximum activity was attained on day 3 of growth, regardless of the presence of the inducer (table).

Biosynthesis of lipids and lipase in the presence and absence of an inducer in the medium

Culture	Medium	Lipids, % dry biomass			
		day 3		day 5	
		1	2	1	2
S. canosus 71	Soybean	9.22 ± 0.04	20.52 ± 0.25	9.35 ± 0.05	22.1 ± 0.1
	M-1	8.72 ± 0.05	9.51 ± 0.04	9.46 ± 0.09	9.1 ± 0.06
S. massasporeus 36	Soybean	4.01 ± 0.05	14.4 ± 0.26	5.18 ± 0.09	13.57 ± 0.07
	M-1	6.53 ± 0.07	9.54 ± 0.09	11.98 ± 0.13	25.46 ± 0.13
Culture	Medium	Lipase, U/mg protein			
		day 3		day 5	
		1	2	1	2
S. canosus 71	Soybean	12.85 ± 0.27	14.44 ± 1.45	4.03 ± 0.08	3.07 ± 0.27
	M-1	0.5 ± 0.0	1.07 ± 0.05	0	0
S. massasporeus 36	Soybean	19.38 ± 2.9	61.88 ± 2.29	0	6.0 ± 0.0
	M-1	3.2 ± 0.15	5.38 ± 0.44	0.17 ± 0.0	0.61 ± 0.01

Note: 1 and 2 correspond, respectively, to the absence and presence of the inducer.

Comparative analysis of the data obtained allowed us to establish a certain relationship between the biosyntheses of lipids, lipase, and LOG (Figs. 1 and 2). An increase in the lipid content, paralleled by a decrease in the lipase and LOG activity, took place over time as the cultures grew older. This effect was characteristic of organisms cultured in both media (soybean medium and medium M-1).

The lipolytic enzymes forming fatty acid free radicals from membrane lipids activated LOG. In the presence of high amounts of esterified fatty acids, the activity of the enzyme was low; free fatty acids stimulated the activity [4]. This circumstance may account for the observation that the content of lipids (among which esterified fatty acids were predominant) increased on day 5, when the LOG and lipase activities were low. The high activities of LOG and lipase, observed on day 3, are interrelated: the cleavage of lipids by lipase supplied the substrates (free fatty acids) for LOG.

There is evidence that changes in the amount of lipid hydroperoxides accumulated in the course of culturing are intimately related to processes of biomass accretion [2]. Hydroperoxides, formed in LOG-catalyzed reactions, are toxic to cells. When a LOG and a lyase act in a concert, hydroperoxides are converted into growth and development stimulators [3, 4]. Accumulation of hydroperoxides probably suppresses LOG activity, which may account for our observation that the enzyme activity declines as the cultures grow.

Thus, there is a certain interrelation between the biosyntheses of lipids, LOG and lipase in streptomycetes. High activity of LOG and lipase were detected on day 3 of culture growth, when the content of lipids fell to minimum. Attenuation of the activities of these enzymes, which was observed as the cultures grew older, was paralleled by an increase in the lipid content.

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