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EXPERIMENTAL ARTICLES

Growth-Coupled Lipid Synthesis in *Mortierella alpina* LPM 301, a Producer of Arachidonic Acid

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Abstract—*Mortierella alpina* LPM 301, a producer of arachidonic acid (ARA), was found to possess a unique property of intense lipid synthesis in the period of active mycelium growth. Under batch cultivation of this strain in glucose-containing media with potassium nitrate or urea, the bulk of lipids (28–35% of dry biomass) was produced at the end of the exponential growth phase and remained almost unaltered in the stationary phase. The ARA content of lipids comprised 42–50% at the beginning of the stationary phase and increased continuously after glucose depletion in the medium due to the turnover of intracellular fatty acids; by the end of fermentation (189–210 h), the amount of ARA reached 46–60% of the total fatty acids (16–19% of dry mycelium). Plausible regulatory mechanisms of the growth-coupled lipid synthesis in microorganisms are discussed.

Key words: lipids, growth, arachidonic acid, *Mortierella alpina.*

Active lipid synthesis in microorganisms is typically a two-phase process proceeding after the cessation of cell growth. Information is available on only a few yeast strains that possess the unique capability of growth-coupled lipid synthesis [1–5]. Mechanisms responsible for the regulation of active lipid synthesis coupled with cell growth are so far unknown.

There has recently been increasing interest in the microbiological production of arachidonic acid (ARA). Owing to its high physiological activity, ARA has found wide application in medicine, pharmacology, cosmetics, the food industry, agriculture, and other fields. The fungi of the genus *Mortierella*, belonging to the class *Phycomycetes*, have been considered as promising producers of ARA [6–8]. Upon screening of more than 100 strains of the genus *Mortierella*, we have earlier revealed ARA production in 65 strains belonging to 32 species [9, 10]. Strain *Mortierella alpina* LPM 301 was selected as a highly active producer of ARA.

The aim of the present work was to study the synthesis of lipids and ARA by *M. alpina* LPM 301 under batch cultivation in glucose-containing media with potassium nitrate or urea as the nitrogen source.

MATERIALS AND METHODS

Strain *M. alpina* LPM 301 was obtained from the culture collection of the Laboratory of Physiology of Microbial Culture Growth at the Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences. The fungus was cultivated in a 10-l ANKUM-2M fermentor (Russia) with a working volume of 6 l in urea-containing medium or in a 100-l BIOR fermentor (Russia) with a working volume of 60 l in medium with potassium nitrate. The media contained (g/l) glucose, 66.0; KH_2PO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.5; yeast extract (Difco, United States), 2.5; KNO_3, 6.0 or urea, 1.5; tap water, 1 l. The temperature (25 ± 0.5°C) and pH (6.0 ± 0.1) were maintained automatically during fermentation.

Biomass concentration was assayed gravimetrically; glucose was analyzed spectrophotometrically [11]. To determine fatty acid composition, the mycelium was vacuum-dried at 70°C to a constant weight and subjected to methanolysis [12]. Fatty acid methyl esters were analyzed by gas-liquid chromatography on a Chrom-5 chromatograph (Czech Republic) equipped with a flame-ionization detector and a glass column (200×0.3 cm) packed with 15% Reoplex-400 on Chromaton N-AW (0.16–0.20 mm) at a column temperature of 200°C; argon was used as the carrier gas. Total lipids were calculated as the sum of fatty acids; heptadecanoic acid or *n*-docosane was used as an internal standard.

RESULTS AND DISCUSSION

The fungi of the order *Mucorales* are usually cultivated on complex media containing peptone, potato hydrolysate, or vegetable oils [7, 13, 14]. In preliminary studies, we developed glucose-containing media with potassium nitrate or urea as a nitrogen source that ensured good growth of *M. alpina* LPM 301 and the production of lipids with a high ARA content.

The time courses of *M. alpina* LPM 301 growth, glucose uptake, and lipid production in the media with potassium nitrate or urea are shown in Figs. 1a and 1b,

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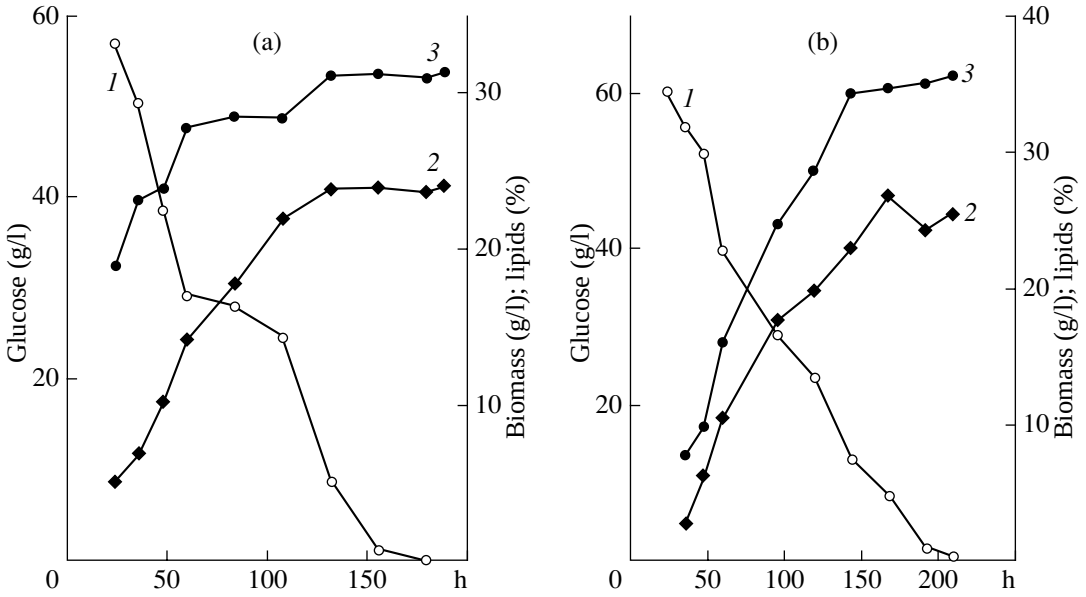

Fig. 1. Growth of *M. alpina* LPM 301 and lipid accumulation in media with (a) potassium nitrate and (b) urea: (*1*) glucose; (*2*) biomass; (*3*) lipids.

respectively. Special attention is attached to the fact that in both media high lipid accumulation coincided with active culture growth. In the potassium nitrate-containing medium, the bulk of lipids (27.7% of dry biomass) was produced by the 60th hour; then, the lipid content of biomass increased only slightly, amounting to 31.1% of dry biomass at the end of fermentation (189 h). In urea-containing medium, the time course of lipid synthesis coincided with that of the culture growth (Fig. 1b); lipid accumulation reached a plateau prior to the stationary phase (by the 144th hour), when the residual glucose concentration remained sufficiently high (13.1 g/l).

Lipid synthesis in oleaginous microorganisms is known to occur mainly in the second phase of batch culture growth under conditions of limitation or inhibition by some component (or factor), when excessive carbon substrate is still available in the medium. Only a few yeast strains were reported to be able to produce great amounts of lipids concurrently with active cell growth, namely, *Cryptococcus terricolus* [1, 2], *Leucosporidium gelidum* [3], and *Debaryomyces globosus* [4, 5]. The capability of *M. alpina* LPM 301 for growth-coupled lipid synthesis is also illustrated by the data on continuous cultivation of this strain; lipid content of biomass increased 1.2-fold with an increase in the specific growth rate from 0.03 to 0.05 h^{-1} [15], whereas oleaginous microorganisms are typically characterized by a negative correlation between the specific growth rate and the level of lipid accumulation [16].

Mechanisms responsible for the regulation of growth-coupled active lipid synthesis in microorganisms are unclear. A biochemical explanation for the lipid accumulation in the second phase of batch culture growth was suggested by Ratledge and coworkers [2, 17–20]. It was stated that the depletion of nitrogen in the growth medium evokes a decrease in the intracellular concentration of AMP, an activator of isocitrate dehydrogenase, and, as a consequence, an increase in the contents of citrate and isocitrate in the cells. Since glucose uptake by the cells continued under nitrogen limitation, citrate became a major metabolite. Citrate is converted by ATP : citrate lyase to oxaloacetate and acetyl-CoA; the latter is used for the synthesis of fatty acids. ATP : citrate lyase is considered to play a key role in the fatty acid synthesis: it has been revealed only in oleaginous microorganisms [19].

It may be assumed that growth-coupled active lipid synthesis in several unique strains of oleaginous microorganisms involves an impaired regulation of the tricarboxylic acid cycle, intense citrate accumulation, and enhanced activity of ATP : citrate lyase during active growth of the producer.

Intense lipid synthesis in microorganisms may be considered as a combination of growth-associated processes (formation of functional lipids, mainly, phospholipids) and processes unessential for cell growth (accumulation of storage lipids, mainly, triacylglycerols) [21]. It seems quite reasonable to assume that ARA, which exhibits a high physiological activity, is a component of functional lipids and, therefore, its synthesis should proceed concurrently with mycelium growth. As can be seen from the data presented in Tables 1 and 2, the level of ARA in lipids continuously increased during growth of *M. alpina* LPM 301 in urea- or nitrate-containing media and reached 42–50% of the total fatty acids (15% of dry mycelium) at the beginning of the stationary phase. However, membrane phos-

Table 1. Fatty acid composition of lipids in the course of *M. alpina* LPM 301 growth in medium with potassium nitrate

Time, h	Acids, % of the total fatty acids													
	14 : 0	15 : 0	16 : 0	18 : 0	18 : 1	18 : 2	γ-18 : 3	20 : 0	20 : 1	20 : 2	20 : 3	20 : 4	22 : 0	24 : 0
24	1.5	0.6	16.8	9.0	19.8	21.2	4.9	0.4	0	0	3.9	19.9	0.4	0
36	1.5	0.4	16.7	10.6	17.0	17.5	4.9	0.4	0	0.1	3.7	24.4	1.0	0.8
48	1.3	0.2	15.1	11.5	16.1	17.1	4.1	0.4	0	0.2	3.6	28.3	0	1.1
60	1.1	0.2	14.3	11.1	15.5	14.5	4.3	0.4	0	0.2	3.6	32.3	1.2	0.6
72	1.1	0.2	14.3	12.1	15.7	12.6	4.6	0.5	0	0.3	3.4	33.1	1.2	0.8
84	1.0	0.2	13.4	11.5	14.9	11.4	4.0	0.7	0.3	0.3	3.7	35.9	1.4	0.7
108	0.8	0.1	12.4	11.2	13.5	9.9	2.1	0.8	0.3	0.3	3.4	41.2	1.7	0.8
132	0.4	0.1	10.2	9.7	11.6	8.3	3.8	0.8	0.3	0.4	2.6	50.2	1.5	0
156	0.3	0.1	9.2	7.6	10.0	7.6	3.4	0.8	0.3	0.3	0	56.9	2.0	1.4
180	0.2	0.2	8.1	5.9	9.3	9.8	3.2	0.5	0	0.3	0	58.4	2.2	1.6
189	0.2	0.2	7.6	5.7	8.6	8.9	3.0	0.4	0	0.2	0	60.4	2.3	1.9

Table 2. Fatty acid composition of lipids in the course of *M. alpina* LPM 301 growth in urea-containing medium

Time, h	Acids, % of the total fatty acids													
	14 : 0	15 : 0	16 : 0	18 : 0	18 : 1	18 : 2	γ-18 : 3	20 : 0	20 : 1	20 : 2	20 : 3	20 : 4	22 : 0	24 : 0
24	0.3	0.3	11.7	5.5	19.5	33.5	5.1	0.3	0	0	0.1	18.9	2.0	0.4
36	0.7	0.9	13.3	0.1	20.2	33.2	6.8	0.2	0	0.1	2.3	18.1	0.8	0.4
48	0.1	1.5	17.4	1.7	20.2	21.2	6.5	0.3	0	0.1	3.9	23.1	0.5	1.4
60	1.6	0.4	16.9	9.0	16.8	13.5	5.6	0.3	0	0.3	4.5	27.6	1.3	1.3
72	1.7	0.3	17.0	9.2	18.4	12.5	4.8	0.5	0.3	0.1	3.6	29.0	1.2	1.0
96	1.5	0.2	23.5	2.3	19.4	11.4	4.1	0.7	0.4	0.2	3.2	30.0	1.3	1.0
120	1.0	0.2	14.8	9.9	17.0	10.8	4.2	0.8	0.4	0.2	2.8	34.8	1.3	1.3
144	0.7	0.1	13.5	9.9	16.3	10.5	3.6	0.6	0.4	0.3	2.7	38.7	1.4	1.1
168	0.5	0.2	12.0	8.8	15.1	10.4	4.0	0.6	0.4	0.3	2.8	42.2	1.5	0.9
192	0.5	0.2	11.7	8.8	14.5	10.6	3.8	0.7	0.4	0.4	2.8	43.0	1.4	1.0
210	0.4	0.2	10.8	8.2	13.6	10.2	3.4	0.7	0.4	0.5	2.7	46.0	1.6	1.2

pholipids obviously cannot contain such large amounts of ARA. Our earlier studies with *Mortierella hygrophila* revealed the occurrence of ARA not only in phospholipids, but also in about 60% of triacylglycerol molecules [22]; it was suggested that the metabolic role of triacylglycerols in fungi is not restricted to the function of energy reserve. It should be noted that ARA synthesis in *M. alpina* LPM 301 persisted after the cessation of mycelium growth. It is obvious that the observed increase in the level of ARA in lipids in the stationary phase, when the lipid content of the biomass remained constant, could have occurred only due to the metabolism of other intracellular fatty acids. As can be seen from Tables 1 and 2, the ARA content of lipids in the stationary phase increased by 7–10%, mainly due to a decrease in the amounts of saturated fatty acids and oleic acid. In this case, the content of direct precursors of ARA (linoleic and γ-linolenic acids) changed insignificantly, possibly, because of their active conversion to ARA. At the end of fermentation (189–210 h), the amount of ARA reached 46–60% of the total fatty acids (16–19% of dry biomass).

To conclude, it should be emphasized that strain *M. alpina* LPM 301, due to its unique property of growth-coupled lipid synthesis, may be considered a promising producer of ARA under continuous cultivation.

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