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INTENSIFICATION OF SURFACTANT SYNTHESIS OF *ACINETOBACTER CALCOACETICUS* IMV B-7241 ON ETHANOL IN PRESENCE OF ORGANIC ACIDS

In the previous researches it was shown that the *Acinetobacter calcoaceticus* IMV B-7241 strain isolated from oil-contaminated soil samples synthesized the surface-active substances (SAS) while growing on hydrophilic and hydrophobic substrates [1]. The conditions of IMV B-7241 strain cultivation on ethanol providing maximum rates of surfactant synthesis were set.

Nowadays, the world's main raw materials for the surfactant synthesis are hydrophobic substrates (usually *n*-hexadecane and liquid paraffin). It should be noted that ethanol is much cheaper and more technological substrate comparing with hydrophobic water insoluble compounds. The use of ethanol for the surfactant biosynthesis can reduce the cost of cultivation but the yield of the desired product remains low.

One of the approach to improve the efficiency of microbial technology is to introduce exogenous biosynthesis precursors into the medium – intermediate products of metabolism of the growth substrate (primary metabolites), which is the source for the processes of constructive metabolism or synthesis regulators (inductors) of the target product.

Thus, it was previously shown that introduction of 0.2% fumarate (precursor of gluconeogenesis) and 0.1% citrate (regulator of lipid synthesis) at the beginning of the stationary growth phase of *Rhodococcus erythropolis* EK-1 on ethanol was accompanied by the increase of surfactant synthesis by 40–100% comparing with

bacteria growing on medium without fumarate and citrate [2]. The increasing of surfactant synthesis under these conditions is due to the strengthening of gluconeogenesis that was confirmed by the increase in 1.5 and 3.5 fold of the isocitratelyase and phosphoenolpyruvate (PEP)-synthetase activity (the key enzymes of glyoxylate cycle and gluconeogenesis, respectively), and also lipid synthesis, that could indicate the 1.5-fold reduction of isocitrate dehydrogenase activity [2]. The increase of surfactant concentration by 1.5–1.7-fold in the case of introducing into the nutritive medium of *R. erythropolis* EK-1 with *n*-hexadecane fumarate 0.2% and 0.1% citrate was caused by the intensification of trehalose mycolates synthesis that was confirmed by the 3–5-fold increase of the activity of PEP-synthetase and trehalose phosphate synthase, respectively [3].

As surfactant synthesized by *A. calcoaceticus* IMV B-7241 on ethanol is complex of glyco-, amino- and neutral lipids [1], we assumed that the introduction of fumarate and citrate into the medium as well as for the strain of *R. erythropolis* EK-1 might be accompanied with the increase of the surfactant biosynthesis.

The aim of the work was to study the possibility of intensifying the synthesis of surfactants *A. calcoaceticus* IMV B-7241 on ethanol in the presence of fumarate and citrate.

At the first stage, similarly to the researches conducted with *R. erythropolis* EK-1 [2, 3], we studied the influence of precursors (0.1–0.5%) on the surfactant synthesis in the case of introducing them into the medium with ethanol in the late exponential and the beginning of the stationary growth phase of *A.calcoaceticus* IMV B-7241. It was determined that the introduction of fumarate and citrate hardly affected the synthesis of surfactants. In this regard, at the next stage of the research the concentration of fumarate and citrate was reduced and organic acids were introduced into the ethanol containing medium at the beginning of the process of *A. calcoaceticus* IMV B-7241 cultivation or in the late exponential growth phase. It is shown that the highest rates of surfactant synthesis were observed at the

simultaneous introduction of fumarate and citrate in the 0.01% concentration (table).

Synthesis of surfactants on the conditions of *A. calcoaceticus* IMV B-7241 growth on ethanol at the presence of different concentrations of fumarate and citrate

Concentration of organic acids*	Surfactant concentration, g/L	Emulsification index**, %
Citrate, 0.01	2.6±0.13	91±4
Citrate, 0.02	2.6±0.13	100±5
Citrate, 0.1	1.9±0.10	87±4
Fumarate, 0.01	2.8±0.14	100±5
Fumarate, 0.02	2.5±0.12	87±4
Fumarate, 0.1	2.1±0.10	88±4
Citrate, 0.01 + Fumarate, 0.01	5.0±0.25	89±4
Citrate, 0.02 + Fumarate, 0.02	3.2±0.16	79±4
Citrate, 0.1 + Fumarate, 0.1	2.8±0.14	88±4
Control (without organic acids)	1.7±0.09	88±4

* The introduction of citrate and fumarate was conducted in the late exponential growth phase (48 h).

** Substrate for emulsification – is the sunflower oil.

Thus, the addition of 0.01 % of organic acids in the substrate with ethanol in the late exponential growth phase *A. calcoaceticus* IMV B-7241 was accompanied by the increasing of the concentration of synthesized surfactants almost in 3 times (from 1.7 to 5.0 g/L) comparing with the cultivation of bacteria in the medium without fumarate and citrate (table). In the case of adding of organic acids in

concentration 0.02% the increase of surfactant synthesis to 3.2 g/L was observed (almost two times higher than in the medium without organic acids). It should be noted that unlike *R. erythropolis* EK-1 [2, 3], in the process of the cultivation of *A. calcoaceticus* IMV B-7241 either on ethanol or on ethanol in the presence of fumarate and citrate, the emulsification index of culture liquid was almost unchanged.

At the next stage the activity of enzymes of surfactant biosynthesis with the introduction into a medium with ethanol organic acids at concentration 0.01% was analyzed. The experiments have shown that under such conditions the 1.7–7.0-fold increase of the activity of all enzymes was obtained excepting isocitratelyase the activity of which was almost the same. The increase of PEP-synthetase and PEP-carboxylase activity (more than 7 and 2.4 fold compared with the activity in the medium with ethanol without organic acids) was the most significant. These results may be evidence of the strengthening of synthesis of surfactant glycolipids in such conditions of strain IMV B-7241 cultivation. The proof of this was more than 3-fold increase of the trehalose phosphate synthase activity – a key enzyme of the biosynthesis of trehalose mycolates. The increasing of isocitrate dehydrogenase and $NADP^+$-dependent glutamate dehydrogenase activity and 2-oxoglutarate dehydrogenase absence in the process of growth strain IMB B-7241 on ethanol in the presence of fumarate and citrate may indicate the increasing of aminolipids synthesis.

The patterns of precursors biosynthesis influence on the formation of *A. calcoaceticus* IMV B-7241 surfactant were determined. They differed from those for the strain *R. erythropolis* EK-1 [2]: firstly, the optimum concentration of fumarate and citrate for the strain IMV B-7241 was 10-fold lower; secondly, the presence of organic acids increased only the synthesis of surfactant and thirdly, the effect of simultaneous use of organic acids in the nutritive medium with ethanol pronounced – the concentration of surfactant increased almost by three folds, while for the strain *R. erythropolis* EK-1 it increased only twice.

Our results also differ from those ones in the literature. Firstly, the literature describes the increase in surfactant synthesis in the presence of citrate only, which was introduced into the nutritive medium at the beginning of the cultivation [4, 5]. Secondly, the optimal concentration of citrate was 0.5–1.0%. With such concentration citrate can be seen not as a regulator of lipid synthesis but as the additional growth substrate. It should be noted that we have not found the information about the intensification of surfactant synthesis by simultaneous introduction into the medium either citrate or C_4-dicarboxylic acids.

The obtained results testify to the possibility of the regulation of surfactant biosynthesis processes in *A.calcoaceticus* IMV B-7241 and changing their orientation toward the formation of surface-active substances.

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