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GLYCEROL METABOLISM IN PRODUCERS OF SURFACE-ACTIVE SUBSTANCES *RHODOCOCCUS ERYTHROPOLIS* IMV Ac-5017, *ACINETOBACTER CALCOACETICUS* IMV B-7241 AND *NOCARDIA VACCINII* K-8

Fast increase of biodiesel production has created an excess of technical glycerol (a by-product of etherification of vegetable oils and animal fats) that in turn led to decreasing of prices on this product in 10 times only for recent years and the growing need for its disposal. The alternative solution of this problem is utilization of glycerol in the technologies of microbial synthesis of practically valuable products, including the surface-active substances (SAS) [1, p.30]. Unique properties of microbial surfactants enable their application in various industries and environmental biotechnology [2, p.427].

In our present work it was shown the principal possibility of intensification of SAS synthesis by *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vaccinii* K-8 on the mixture of glycerol and hexadecane. However, to secure maximum conversion of carbon into the target product it is necessary to determine the optimal for its synthesis molar ratio of monosubstrates in the mixture, which in turn requires theoretical calculations of the energy needs for SAS and biomass synthesis [3, p.145]. So long as for making such calculations it is need to know ways of metabolism of corresponding

monosubstrates, the aim of this work was to study features of glycerol metabolism in the producers of SAS *R. erythropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vaccinii* K-8.

Glycerol can be utilized as a carbon source by bacteria via several metabolic pathways that convert glycerol to dihydroxyacetonephosphate (DHAP) before DHAP enters the glycolytic pathway. [4, p. 780; 5, p.716]. The first pathway begins with the ATP-dependent phosphorylation of glycerol by glycerolkinase, and glycerol-3-phosphate then is oxidized to DHAP by glycerol-3-phosphatedehydrogenase or glycerol-3-phosphateoxidase (glycerol-3 -phosphate pathway). The second pathway begins with oxidation of glycerol to dihydroxyacetone by nicotinamide adenine dinucleotide (NAD^+) or pyrroloquinoline quinone (PQQ) – dependent glyceroldehydrogenase. Dihydroxyacetone is phosphorylated to DHAP by dihydroxyacetonekinase (dihydroxyacetone pathway).

In cell-free extracts of *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* K-8 it was identified the activity of NAD^+-dependent glyceroldehydrogenase and no activity of PQQ-dependent. As long as in the previous studies [6, p.23] it was shown the broad substrate specificity of N,N-dimethylnitrosamine (NDMA)-dependent alcohol dehydrogenase of *A.calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017, the next step was to investigate the role of these enzymes in the glycerol oxidation by studied strains. As a result it was found that oxidation of glycerol in strains IMV B-7241, IMV Ac-5017 and K-8 is catalyzed by two enzymes: PQQ-dependent glyceroldehydrogenase and NDMA-dependent alcohol dehydrogenase (Table 1).

Analysis of enzymes of glycerol-3-phosphate pathway of glycerol catabolism in all three strains showed rather high activity of glycerolkinase and NAD^+-dependent glycerol-3-phosphatedehydrogenase, but not the activity of FAD^+-dependent glycerol-3-phosphatedehydrogenase (Table 1).

Table 1

Activity of enzymes of glycerol catabolism pathways in *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* K-8

Pathways	Enzymes	Activity (nmol· min^{-1}· mg^{-1} of protein)		
		IMV B-7241	IMV Ac-5017	K-8
Dihydroxy-acetone	PQQ-dependent glyceroldehydrogenase	107,5±5	94,5±4	256±13
	NDMA-dependent alcoholdehydrogenase	32±1,6	24±1,2	550±28
	Dihydroxyacetonekinase	336±16	288±14	732±37
	NAD^+-dependent glyceroldehydrogenase	0	0	0
	PQQ-dependent alcohol dehydrogenase	0	0	0
Glycerol-3 -phosphate	Glycerolkinase	780±39	800±40	244±12
	NAD^+- dependent glycerol-3-phosphate dehydrogenase	159±8	108±5	488±24
	FAD^+-dependent glycerol-3-phosphate dehydrogenase	0	0	0

In Table 2 it is shown data on the activity of enzymes of biosynthesis of surface-active amino – (glutamatede hydrogenase) and glycolipids (phosphoenolpyruvate (PEP)-carboxykinase, PEP-synthase) and anaplerotic reactions (isocitrate lyase and PEP-carboxylase) under the growth of strains IMV B-7241, IMV Ac-5017 and K-8 in medium with glycerol. It should be mentioned that in such conditions in *A. calcoaceticus* IMV B-7241 and *N. vaccinii* K-8 glyoxylate cycle does not work (no activity of isocitrate lyase was detected) and refill of the pool of C_4-dicarboxylic acids occurs in PEP- carboxylase reaction. Unlike the other two strains of *R. erythropolis* IMV Ac-5017 it was found low

isocitrate lyase activity, so it is obvious that in glycerol-growing strain IMV Ac-5017 the main anaplerotic reaction is also a reaction catalyzed by PEP-carboxylase (Table 2). Revealed very high activity of key enzymes of gluconeogenesis indicates the biosynthesis of glycolipids from glycerol by all three strains, and the glutamate dehydrogenase activity - also the formation of surface-active aminolipids by strains IMV B-7241 and K-8. From the data obtained we can conclude that the major components of SAS formed by *R. erythropolis* IIR Al-5017 and *A. calcoaceticus* IMV B-7241 during the growth on glycerol did not differ from those, obtained after cultivation of strains on ethanol [7, p.472; 8, p. 275].

Table 2

Activity of enzymes of the SAS biosynthesis during the growth of *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* K-8 on glycerol

Enzymes	Activity (nmol· min^{-1}· mg^{-1} of protein)		
	IMV B-7241	IMV Ac-5017	K-8
$NADF^{+}$- dependent glutamate dehydrogenase	597±30	not measured	329±16
Isocitrate lyase	0	44±2	0
PEP-carboxylase	1045±52	2727±136	656±33
PEP-synthase	1780±89	2428±121	23667±1183
PEP-carboxykinase	448±22	909±45	820±41

Thus, as a result of the work it was established that the strains IMV B-7241, IMV Ac-5017 and K-8 metabolize glycerol to DHAP via both known pathways. The data obtained could be the basis for theoretical calculations of the energy needs for SAS and biomass synthesis on this substrate to determine the optimal molar ratio of energy unequivalent substrates in order to increase the synthesis of SAS of *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N.*

vaccinii K-8 on their mixture.

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