ISSN 0026-2617, Microbiology, 2007, Vol. 76, No. 6, pp. 698–703. © Pleiades Publishing, Ltd., 2007.
Original Russian Text © T.G. Pirog, N.V. Vysyatetskaya, Yu.V. Korzh, 2007, published in Mikrobiologiya, 2007, Vol. 76, No. 6, pp. 790–796.

EXPERIMENTAL ARTICLES

Formation of the Exopolysaccharide Ethapolan by *Acinetobacter* sp. IMV B-7005 on a Fumarate–Glucose Mixture

T. P. Pirog[1], N. V. Vysyatetskaya, and Yu. V. Korzh

Zabolotnyi Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kiev, Ukraine

Received December 28, 2006

Abstract—Our studies enabled us to intensify the synthesis of the microbial exopolysaccharide (EPS) ethapolan produced by *Acinetobacter* sp. IMV B-7005 grown on a mixture of fumarate (an energy-excessive substrate) and glucose (an energy-deficient substrate). Supplementing glucose-containing medium with sodium (potassium) fumarate at a molar ratio of 4 : 1 resulted in a 1.3–2.2-fold increase of the EPS amount synthesized and in a 1.3–2-fold increase of the EPS yield relative to the biomass compared to cultivation on monosubstrates. The conversion of the carbon of both substrates to EPS was the highest if the carbon/nitrogen ratio in the cultivation medium was 70.5 and inoculum grown on glucose monosubstrate was used.

Key words: energy-excessive substrate, energy-deficient substrate, biosynthesis intensification, exopolysaccharides.

DOI: 10.1134/S0026261707060070

Our recent works have demonstrated the possibility of enhancing the synthesis of the microbial exopolysaccharide ethapolan by *Acinetobacter* sp. IMV B-7005 grown on a mixture of substrates (ethanol + glucose) that are energetically nonequivalent [1–4]. Theoretical calculation of the energy requirements of *Acinetobacter* sp. B-7005 relating to biomass and EPS synthesis on an energy-deficient substrate (glucose) enabled us to determine the "complementary" concentration of the energy-excessive substrate (ethanol). It allowed replenishing the loss of glucose carbon caused by oxidizing glucose to CO_2 to obtain energy for constructive metabolism. The addition of ethanol to the glucose-containing medium at a molar ratio of 3.1 : 1 resulted in an increase in the EPS amount obtained and EPS yield relative to the substrate consumed, compared to cultivation on monosubstrates.

Our data on the characteristics of the energy and constructive metabolism of *Acinetobacter* sp. B-7005 [5] in conjunction with Babel's principles of substrate classification in terms of energetics [6] suggest that fumarate is a potential energy-excessive substrate for the ethapolan producer.

Therefore, the goal of this work was to investigate the characteristics of ethapolan synthesis on a fumarate–glucose mixture.

[1] Corresponding author; e-mail: tapirog@usuft.kiev.ua

MATERIALS AND METHODS

Research subjects. The studies were conducted with the strain of *Acinetobacter* sp. that produces the complex polysaccharide preparation ethapolan [7]. The strain (designated B-7005) is stored at the Depositary of Microorganisms of the Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

Ethapolan consists of an acetylated and an nonacetylated polysaccharide (AP and NAP, respectively). They are similar in terms of the molar ratio of D-glucose, D-mannose, D-galactose, L-rhamnose, D-glucuronic acid, and pyruvic acid (3 : 2 : 1 : 1 : 1 : 1) and the structure of the carbohydrate chain unit. The difference between these EPS is that only AP contains C_{12}–C_{18} fatty acids. These are lauric, palmitic, palmitoleic, stearic, and oleic acid [2].

Cultivation of *Acinetobacter* sp. B-7005 was carried out in flasks at 30°C for 16–96 h in liquid mineral media with shaking (220 rpm). The media composition was as follows (g/l): medium 1: KH_2PO_4, 3.4; KOH, 0.9, NH_4Cl, 0.4; $MgSO_4 \times 7H_2O$, 0.4; $CaCl_2 \times 2H_2O$, 0.1; $FeSO_4 \times 7H_2O$, 0.001. Medium 2: KH_2PO_4, 3.4; NH_4Cl, 0.4; $MgSO_4 \times 7H_2O$, 0.4; $CaCl_2 \times 2H_2O$, 0.1; $FeSO_4 \times 7H_2O$, 0.001. Medium 3: KH_2PO_4, 2.0; NH_4Cl, 0.4; $MgSO_4 \times 7H_2O$, 0.4; $CaCl_2 \times 2H_2O$, 0.1; $FeSO_4 \times 7H_2O$, 0.001. The media were supplemented with 0.5% yeast autolysate (vol/vol) and 0.0009% calcium pantothenate (vitamin B_5). The strain *Acineto-*

bacter sp. B-7005 is auxotrophic for this vitamin. The carbon and energy sources used by us were:

(i) 1.85% glucose as a monosubstrate;

(ii) 2.5% sodium fumarate or 2.9% potassium fumarate, respectively, as monosubstrates;

(iii) a mixture of 0.9, 1.35, 1.8, 2.25, or 2.7% sodium fumarate and 0.5% glucose at molar ratios of 2 : 1, 3 : 1, 4 : 1, 5 : 1, or 6 : 1, respectively; and

(iv) a mixture of 0.5% glucose and 2.1% potassium fumarate.

The concentrations of monosubstrates are equimolar in terms of carbon content to those of mixed substrates if the molar ratio between fumarate and glucose is 4 : 1 (1.8% sodium fumarate + 0.5% glucose or 2.1% potassium fumarate + 0.5% glucose). The tested sodium fumarate and potassium fumarate concentrations are equimolar in terms of carbon content. In one of the studies, we varied the glucose concentration in the mixed substrate (0.25, 0.75, and 1.0%), while the sodium fumarate concentration was constant (1.8%). The molar ratios between the fumarate and glucose concentrations were 5 : 1, 3 : 1, and 2 : 1, respectively.

In the experiments concerning the dependence of ethapolan synthesis on the ratio between the fumarate and glucose concentrations, medium 3 was used for the cultivation. The carbon/nitrogen ratio varies from 45 to 96 depending on the substrate concentrations in the cultivation medium. In one of the experiments, this ratio was therefore maintained at a level of 70.5 that is optimum for ethapolan synthesis.

Exponential-phase (18–24 h) cultures grown on media 1, 2, or 3 were used as inocula. Glucose (0.5%), potassium fumarate (0.8%), and sodium fumarate (0.7%) were employed as carbon sources in the cultivation medium for the inoculum.

Growth and EPS synthesis parameters including dry biomass (DB), EPS concentration, EPS-synthesizing capacity, and EPS yield relative to the substrate consumed were determined as described in [1].

Glucose concentration in the culture liquid was determined by the glucose oxidase method [8]. Fumarate concentration was determined by the Stotz method [9].

The energy consumption for the synthesis of biomass and EPS. The energy consumption for biomass synthesis was determined as described in [6]. The energy requirements related to EPS synthesis were determined according to [2, 10].

Three repeats of all experiments were conducted. The statistical treatment of the results was based on the Student test with a significance level of 5%.

RESULTS AND DISCUSSION

Calculating the Fumarate–Glucose Concentration Ratio during the Cultivation of Acinetobacter sp. B-7005 on a Fumarate–Glucose Mixture

In order to determine the optimum ratio of concentrations of nonequivalent substrates in the cultivation medium of the ethapolan producer, calculation of the energy requirements relating to biomass and EPS synthesis on an energy-deficient substrate is required. It is also necessary to determine the concentration of the energy-excessive substrate that replenishes the loss of the carbon of the energy-deficient substrate during its oxidation to CO_2 for the purpose of obtaining energy for constructive metabolic processes [2].

The optimum ratio of fumarate and glucose concentrations for cultivating strain B-7005 on a fumarate–glucose mixture was calculated making the following assumptions [2]:

(i) fumarate is predominantly utilized as an energy source, while glucose carbon is utilized for synthesizing biomass and EPS;

(ii) glucose is partially (50%) catabolized via the Embden–Meyerhof–Parnas pathway and the other 50% via the Entner–Doudoroff pathway;

(iii) the P/O ratio is 2;

(iv) the EPS contains 50% of AP and 50% of NAP;

(v) each repeating AP unit contains residues of two fatty acids (lauric, $C_{12}H_{24}O_2$, and palmitic, $C_{16}H_{32}O_2$); and

(vi) the NADPH formed via fumarate and glucose catabolism is the source of the reducing equivalents that are oxidized to water in the respiratory chain.

ATP demand for ethapolan synthesis from glucose. The calculation of the energy requirements for ethapolan synthesis was carried out similarly to that presented in [2]. ATP is consumed for monosaccharide and fatty acid synthesis and is formed during the synthesis of pyruvic acid (PA), glucuronic acid, and acetyl-CoA (the fatty acid precursor) [2].

The energy requirements associated with AP and NAP synthesis (per mol of glucose) are given in Table 1.

According to the calculations carried out in [2], the energy obtained during ethapolan synthesis amounts to 1.42 mol of ATP per mol of glucose.

Energy requirements related to biomass synthesis. Biomass synthesis from phosphoglycerate (PGA) involving ammonium as a nitrogen source proceeds according to the following equation [6]:

$$4\text{PGA} + \text{NH}_3 + 29\text{ATP} + 5.5\text{NADPH} \longrightarrow (\text{C}_4\text{H}_8\text{O}_2\text{N}_1)_3, \quad (1)$$

where $(C_4H_8O_2N_1)_3$ is the molar formula of the biomass.

Table 1. Energy requirements for the synthesis of acetylated and nonacetylated polysaccharides by *Acinetobacter* sp. B-7005 from glucose

Glucose catabolism pathway	EPS	Glucose consumption for EPS unit synthesis mol	Energy costs, mol of ATP		Energy generation, mol of ATP	
			Per EPS unit	Per mol of glucose	Per EPS unit	Per mol of glucose
Embden–Meyerhof–Parnas pathway	AP	15.5	29	1.87	77	4.97
	NAP	8.5	17	2	7	0.12
	AP + NAP	24	46	1.92	84	3.5
Entner–Doudoroff pathway	AP	15.5	29	1.87	69.5	4.48
	NAP	8.5	17	2.0	6.5	0.76
	AP + NAP	24	46	1.92	76	3.17

The overall process of converting glucose to PGA (at a P/O ratio of 2) follows the equation [2, 6]:

$$C_6H_{12}O_6 \longrightarrow 2PGA + 3ATP. \quad (2)$$

Taking into account the enzymological studies on *Acinetobacter* sp. B-7005 [5], we can write the following equation for the overall process of fumarate–PGA conversion (at a P/O ratio of 2):

$$C_4H_4O_4 \longrightarrow PGA + 4ATP + CO_2. \quad (3)$$

From the equations of biomass synthesis from PGA (equation 1) and glucose catabolism to PGA (equation 2) it ensues that the ATP demand (per mol of glucose) for biomass synthesis while cultivating *Acinetobacter* sp. B-7005 on glucose is 17 mol. We believe that this energy can be obtained from fumarate. The amount of ATP formed from fumarate should be 17 – 14.2 = 15.58 mol, because EPS synthesis from glucose yields 1.42 ATP per 1 mol of glucose. It follows from equation 3 that obtaining this ATP amount requires 3.9 mol of fumarate. Accordingly, the molar ratio of fumarate and glucose in the medium should be 3.9 : 1 (4 : 1). For example, at a glucose concentration of 0.5% (5 g/l, or 0.028 M), this ratio means 0.11 M, i.e. 17.6 (18) g/l sodium fumarate or 21.1 (21) g/l potassium fumarate.

Dependence of Ethapolan Synthesis on the Method of Inoculum Preparation and Medium Composition (Cultivation on the Fumarate–Glucose Mixture)

Our studies provided evidence that growing *Acinetobacter* sp. B-7005 on a sodium fumarate–glucose mixture does not result in catabolic repression; both substrates are consumed simultaneously (Fig. 1). Fig. 1 demonstrates the pattern of changes in sodium fumarate and glucose concentrations during the growth of *Acinetobacter* sp. B-7005 at a sodium fumarate–glucose ratio of 4 : 1 (theoretical calculations). It was

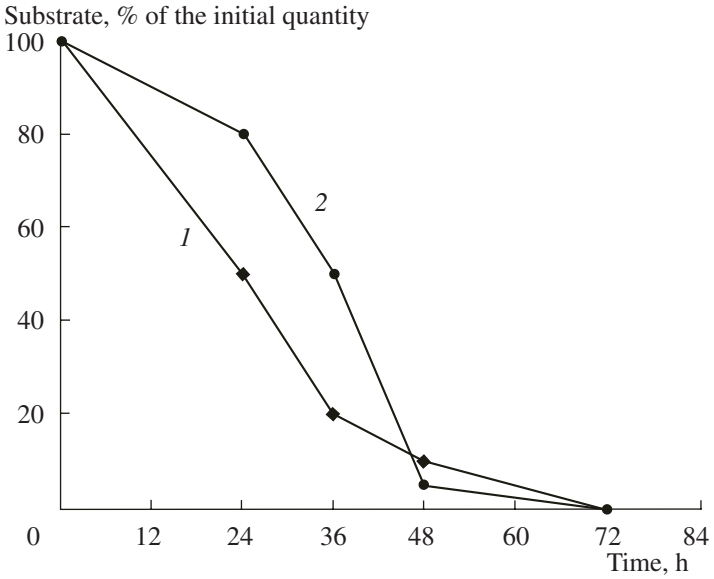

Fig. 1. Consumption of sodium fumarate (*1*) and glucose (*2*) during the cultivation of *Acinetobacter* sp. B-7005 on a mixture of these substrates. Concentrations, %: sodium fumarate 1.8; glucose 0.5. Cultivation was carried out on medium 1.

Table 2. Ethapolan synthesis parameters during the cultivation of *Acinetobacter* sp. B-7005 on glucose, sodium fumarate, and their mixture

Carbon source for EPS biosynthesis, %	Carbon source in the inoculum medium, %	Process parameters		
		Dry biomass (DB), g/l	EPS, g/l	EPS-synthesizing capacity, g of EPS per g of DB
Glucose, 1.85	Glucose, 0.5	0.53	4.70	8.87
	Sodium fumarate, 0.7	0.90	3.30	3.70
Sodium fumarate, 2.5	Glucose, 0.5	0.40	5.60	14.0
	Sodium fumarate, 0.7	0.40	5.90	14.8
Sodium fumarate, 1.8 + glucose, 0.5	Glucose, 0.5	0.40	7.20	18.0
	Sodium fumarate, 0.7	0.45	7.40	16.4

Note: *Acinetobacter* sp. B-7005 was cultivated on medium 1; the monosubstrate concentrations are equimolar in terms of the carbon content of the mixed substrate.

Table 3. Ethapolan synthesis from a sodium fumarate (1.8%)–glucose (0.5%) mixture by *Acinetobacter* sp. IMV B-7005 on various media

Cultivation medium	Carbon source in the inoculum medium	Process parameters		
		EPS, g/l	EPS-synthesizing capacity, g of EPS per g of DB	EPS yield, % of substrate
1	Glucose, 0.5	7.2	18.0	41
	Sodium fumarate, 0.7	7.4	16.4	42
2	Glucose, 0.5	7.1	17.8	40
	Sodium fumarate, 0.7	6.8	19.4	38
3	Glucose, 0.5	9.5	21.3	53
	Sodium fumarate, 0.7	8.0	17.8	45

Note: Media composition is given in the Materials and Methods section. EPS (1.5–5.0 g/l) was synthesized on media 2 and 3 with monosubstrates (1.85% glucose and 2.5% sodium fumarate); EPS yield relative to substrate (sodium fumarate) consumption was calculated assuming the organic portion of the fumarate molecule $C_4H_2O_4^{2-}$ as the substrate.

established that these substrates are assimilated by *Acinetobacter* sp. B-7005 simultaneously, irrespective of their concentration ratios.

Table 2 contains the data on ethapolan synthesis with sodium fumarate, glucose, or a mixture of these substrates. If the ethapolan producer is cultivated on a mixed substrate, the EPS amount obtained and the EPS-producing capacity are considerably (1.3–2.2 times) higher than those characteristic of the respective monosubstrate. Importantly, this pattern occurred regardless of the inoculum preparation method.

At the end of *Acinetobacter* sp. B-7005 cultivation on medium 1 with sodium fumarate and glucose, the pH value of the culture liquid increased to 9.2–9.4 (fumarate is transported into bacterial cells together with a proton), whereas the pH optimum for ethapolan synthesis is 6.8–7.0 [7]. In this context, we suggested that omitting KOH (the alkaline component of the buffer) from the medium would result in shifting the pH value to the optimum level and, therefore, increasing the EPS amount. From the data of Table 3, it is evident that the EPS synthesis parameters reached their maxima if *Acinetobacter* sp. B-7005 grew on medium 3. This medium lacks KOH, and the KH_2PO_4 content is lowered to 2 g/l. Interestingly, the EPS amount, the EPS-synthesizing capacity, and the EPS yield on medium 3 were higher if the inoculum was grown on glucose than on sodium fumarate (Table 3). In subsequent experiments, we cultivated *Acinetobacter* sp. B-7005 on medium 3 using the inoculum grown on glucose.

We have demonstrated earlier [11] that growing the ethapolan producer on a sodium acetate–glucose mixture resulted in the EPS synthesis parameter values almost two times higher than on a potassium acetate–glucose mixture. In our opinion [11], these results suggest an involvement of sodium cations in building up ion gradients across the membrane. They are prerequi-

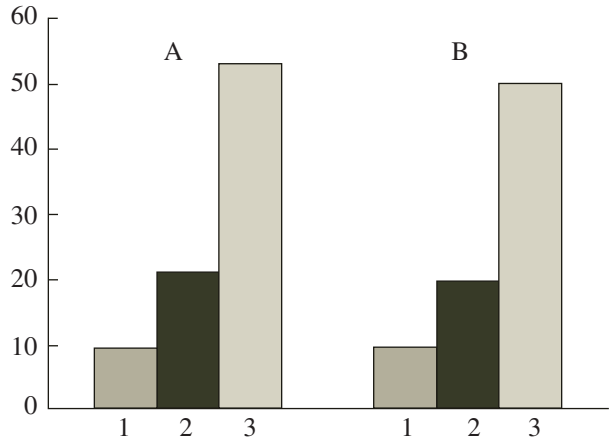

Fig. 2. Ethapolan formation during the cultivation of *Acinetobacter* sp. B-7005 on a glucose–sodium fumarate (A) and a glucose–potassium fumarate (B) mixture. 1, EPS, g/l; 2, EPS-synthesizing capacity (g of EPS per g of DB); 3, EPS yield, % of substrate. Cultivation was carried out on medium 3.

site for generating the proton-motive force used for active acetate transport into *Acinetobacter* sp. B-7005 cells. Active acetate transport was detected by us polarographically, using the protonophore *n*-trifluoromethoxyphenylhydrazone carbonylcyanide (FCCP).

The results given in Fig. 2 indicate that replacing sodium fumarate with potassium fumarate in the mixed substrate had virtually no effect on ethapolan synthesis parameters. Hence, it seems likely that the patterns of acetate and fumarate transport into *Acinetobacter* sp. B-7005 cells are different.

Influence of the Fumarate–Glucose Concentration Ratio on Ethapolan Synthesis

Table 4 contains the results of our research on the dependence of ethapolan synthesis on the sodium fumarate–glucose concentration ratio. The required molar ratio of the substrates was attained by varying sodium fumarate concentration at a constant glucose concentration, or, conversely, by varying glucose concentration at a constant sodium fumarate concentration. The studies demonstrated that the maximum EPS synthesis parameters occur (Table 4) at a fumarate–glucose ratio of 4 : 1 (calculated theoretically). Nevertheless, the results shown in Table 4 are ambiguous: apart from the molar ratio of the substrates, we varied the carbon source concentration and the C/N ratio in the medium (from 45 to 96, Table 4). According to the data reported in the literature, the carbon/nitrogen ratio considerably influences microbial polysaccharide biosynthesis [7]. To rule out the influence of this factor on ethapolan synthesis, in subsequent studies we varied the fumarate–glucose ratio in the medium, maintaining the C/N ratio at a constant level of 70.5. This C/N ratio was chosen for two reasons: (i) it resulted in the highest ethapolan synthesis parameters on a medium containing nonequivalent C_2–C_6 substrates, as shown by us earlier [1], and (ii) we attained a maximum EPS synthesis level on a fumarate–glucose medium at a C/N ratio of 70.5 (Table 4). Table 5 contains the results of our studies concerning the dependence of ethapolan formation on substrate concentration ratio at a constant C/N ratio. The maximum EPS concentration (9.5 g/l) and EPS yield (53%) occurred at a fumarate–glucose ratio of 4 : 1 (calculated theoretically).

We have previously demonstrated [7] that consecutive additions of 0.2% of sodium (potassium) fumarate during the stationary growth phase result in its stoichiometric conversion to EPS during the cultivation of the EPS producer on an ethanol-containing medium. Exogenous fumarate enhances gluconeogenesis in *Acinetobacter* sp. B-7005 cultivated on ethanol. This fact was confirmed by the results of our enzymological studies. It was shown in [5] that the activities of the enzymes of both glyoxylate cycle and gluconeogenesis drastically

Table 4. Influence of the sodium fumarate–glucose molar ratio on ethapolan synthesis

Fumarate–glucose molar ratio	Sodium fumarate concentration, %	Glucose concentration, %	C/N ratio	EPS, g/l	EPS yield, % of substrate
2 : 1	0.9	0.5	44.8	4.1	36
	1.8	1.0	89.5	6.7	29
3 : 1	1.35	0.5	57.6	5.7	39
	1.8	0.75	80.0	8.3	40
4 : 1	1.8	0.5	70.5	9.5	53
5 : 1	2.25	0.5	83.3	8.5	40
	1.8	0.25	60.9	7.3	48
6 : 1	2.7	0.5	96.3	8.2	34

Note: Cultivation was carried out on medium 3; the inoculum was grown on medium 3 with glucose (0.5%); EPS yield relative to substrate (sodium fumarate) consumption was calculated assuming the organic portion of the fumarate molecule $C_4H_2O_4^{2-}$ as the substrate.

Table 5. Ethapolan formation on a sodium fumarate–glucose mixture at various substrate concentration ratios and a constant C/N ratio

Fumarate–glucose molar ratio	Sodium fumarate concentration, %	Glucose concentration, %	EPS, g/l	EPS yield, % of substrat
2 : 1	0.9	0.5	5.7	50
	1.8	1.0	7.8	34
3 : 1	1.35	0.5	7.0	48
	1.8	0.75	8.7	43
4 : 1	1.8	0.5	9.5	53
5 : 1	2.25	0.5	8.9	42
	1.8	0.25	7.9	51

Note: Cultivation was carried out on medium 3; the inoculum was grown on medium 3 with glucose (0.5%); EPS yield relative to substrate (sodium fumarate) consumption was calculated assuming the organic portion of the fumarate molecule $C_4H_2O_4^{2-}$ as the substrate.

increase if an ethanol-containing medium is supplemented with fumarate.

In this work, we revealed that fumarate enhances ethapolan synthesis on a medium with glucose. As an energy-excessive substrate, fumarate compensates for the loss of glucose carbon during its oxidation to CO_2 for the purpose of producing energy for constructive metabolic processes. Hence it increases the efficiency of converting both substrates to EPS. Nonetheless, we cannot rule out the possibility that gluconeogenesis is enhanced by cultivating the ethapolan producer on a medium with fumarate and glucose.

This work demonstrates that the synthesis of the microbial polysaccharide ethapolan can be intensified using a mixture of substrates (glucose + fumarate) that are nonequivalent in terms of energetics. We established the cultivation conditions (the method of inoculum preparation, the ratio of substrate concentrations, and the carbon/nitrogen ratio) that secure the maximum conversion of substrate carbon to EPS.

REFERENCES

1. Pirog, T.P., Kovalenko, M.A., Kuz'minskaya, Yu.V., and Krishtab, T.P., Enhanced Synthesis of the Exopolysaccharide Ethapolan by *Acinetobacter* sp. 12S Grown on a Mixture of Substrates, *Mikrobiologiya*, 2003, vol. 72, no. 1, pp. 26–32 [*Microbiology* (Engl. Transl.), vol. 72, no. 1, pp. 305–312].
2. Pirog, T.P., Kovalenko, M.A, and Kuz'minskaya, Yu.V., Intensification of Exopolysaccharide Synthesis by *Acinetobacter* sp. on an Ethanol-Glucose Mixture: Aspects Related to Biochemistry and Bioenergetics, *Mikrobiologiya*, 2003, vol. 72, no. 3, pp. 348–355 [*Microbiology* (Engl. Transl.), vol. 72, no. 3, pp. 305–312].
3. Pirog, T.P., Kuz'minskaya, Yu.V., and Kovalenko, M.O., Metabolism of C_2–C_6 Substrates by Mixotrophically Grown *Acinetobacter* sp. Strains B-7005 and B-7005 (1NG), *Ukr. Biokhim. Zh.*, 2004, vol. 76, no. 1, pp. 33–38.
4. Pirog, T.P., Kovalenko, M.A., Kuz'minskaya, Yu.V., and Votselko, S.K., Physicochemical Properties of the Microbial Exopolysaccharide Ethapolan Synthesized on a Mixture of Growth Substrates, *Mikrobiologiya*, 2004, vol. 73, no. 1, pp. 19–24 [*Microbiology* (Engl. Transl.), vol. 73, no. 1, pp. 14–18].
5. Pirog, T.P. and Kuz'minskaya, Yu.V., Some Characteristics of Central Metabolism in *Acinetobacter* sp. Grown on Ethanol, *Mikrobiologiya*, 2003, vol. 72, no. 4, pp. 459–465 [*Microbiology* (Engl. Transl.), vol. 72, no. 4, pp. 408–413].
6. Babel, W. and Muller, R.H., Mixed Substrate Utilization in Microorganisms: Biochemical Aspects and Energetics, *J. Gen. Microbiol.*, 1985, vol. 131, no. 1, pp. 39–45.
7. Grinberg, T.A., Pirog, T.P., Malashenko, Yu.R., and Pinchuk, G.E., *Mikrobnyi sintez ekzopolisakharidov na C_1–C_2-soedineniyakh* (Microbial Synthesis of Exopolysaccharides on C_1–C_2 Compounds), Kiev: Nauk. dumka, 1992.
8. Lukomskaya, I.S. and Gorodetskii, V.K., Application of Microcide (Glucose Oxidase) for Determination of Glucose Levels in Normal and Diabetic Blood, *Biokhimiya*, 1961, vol. 26, no. 3, pp. 477–482.
9. Pyatnitskii, M.P. and Yur'eva, A.F., Fumarate Method for Determination of Malic Acid, *Biokhimiya*, 1949, vol. 14, no. 3, pp. 196–200.
10. Jarman, T.R. and Pace, G.W., Energy Requirements for Microbial Exopolysaccharide Synthesis, *Arch. Microbiol.*, 1984, vol. 137, pp. 231–235.
11. Pirog, T.P., Vysyatetskaya, N.V., and Korzh, Yu.V., Specific Features of the Synthesis of the Exopolysaccharide Ethapolan on a Mixture of Energy-Deficient Growth Substrates, *Mikrobiologiya*, 2007, vol. 76, no. 1, pp. 32–38 [*Microbiology* (Engl. Transl.), vol. 76, no. 1, pp. 25–30].