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EXPERIMENTAL ARTICLES

Some Characteristics of Central Metabolism in *Acinetobacter* sp. Grown on Ethanol

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Abstract—Ethanol-grown cells of the mutant *Acinetobacter* sp. strain 1NG, incapable of producing exopolysaccharides, were analyzed for the activity of enzymes of the tricarboxylic acid (TCA) cycle and some biosynthetic pathways. In spite of the presence of both key enzymes (isocitrate lyase and malate synthase) of the glyoxylate cycle, these cells also contained all enzymes of the TCA cycle, which presumably serves biosynthetic functions. This was evident from the high activity of isocitrate dehydrogenase and glutamate dehydrogenase and the low activity of 2-oxoglutarate dehydrogenase. Pyruvate was formed in the reaction catalyzed by oxaloacetate decarboxylase, whereas phosphoenolpyruvate (PEP) was synthesized by the two key enzymes (PEP carboxykinase and PEP synthase) of gluconeogenesis. The ratio of these enzymes was different in the exponential and the stationary growth phases. The addition of the C_4-dicarboxylic acid fumarate to the ethanol-containing growth medium led to a 1.5- to 2-fold increase in the activity of enzymes of the glyoxylate cycle, as well as of fumarate hydratase, malate dehydrogenase, PEP synthase, and PEP carboxykinase (the activity of the latter enzyme increased by more than 7.5 times). The data obtained can be used to improve the biotechnology of production of microbial exopolysaccharide ethapolan on C_2-substrates.

Key words: ethanol metabolism, the tricarboxylic acid cycle, the glyoxylate cycle, gluconeogenesis, biosynthesis.

Acinetobacter sp. 12S is the producer of the complex exopolysaccharide (EPS) ethapolan [1]. To study C_2-metabolism in this bacterium, we derived the EPS-deficient mutant *Acinetobacter* sp. strain 1NG [2], since cells of the parent strain cannot be used in enzymatic studies as their separation from the highly viscous high-molecular-weight EPS is hardly possible. The experimental data obtained earlier showed that the oxidation of ethanol to acetaldehyde in *Acinetobacter* sp. is catalyzed by the NAD^+-linked alcohol dehydrogenase. Acetaldehyde is oxidized by acetaldehyde dehydrogenase with NAD^+ and $NADP^+$ as electron acceptors. Acetate is involved in the metabolism of *Acinetobacter* sp. with the aid of acetyl-CoA synthetase. The presence of isocitrate lyase indicated that the glyoxylate cycle in *Acinetobacter* sp. functions as the anaplerotic sequence of reactions replenishing the cellular pool of C_4-dicarboxylic acids.

The aim of this work was to gain further insight into the C_2-metabolism of *Acinetobacter* sp. by measuring the activity of enzymes of the tricarboxylic acid (TCA) cycle and some biosynthetic pathways, in particular, gluconeogenesis.

MATERIALS AND METHODS

The EPS-deficient *Acinetobacter* sp. strain 1NG used in this study was described earlier [2].

Cultivation conditions. The strain was grown in a liquid medium containing (g/l) KH_2PO_4, 3.4; KOH, 0.9; NH_4NO_3, 0.3; $MgSO_4 \cdot 7H_2O$, 0.4; $CaCl_2 \cdot 2H_2O$, 0.1; $FeSO_4 \cdot 7H_2O$, 0.001; yeast autolysate, 5; and calcium pantothenate, 0.0009% (pH 6.8–7.0). The source of carbon and energy was either 1 vol % ethanol or 1 vol % ethanol + 0.2% potassium fumarate. The latter substrate was added to the medium in the form of a 10% solution. In one of the experiments, in which *Acinetobacter* sp. 1NG was grown on the mixture of ethanol and fumarate, the medium did not contain pantothenate.

The medium was inoculated either with a 1-day-old culture grown on a mixture of nutrient–wort (1 : 1) agar or with an exponential-phase (16- to 18-h-old) culture grown on 0.5 vol % ethanol. The inoculum size was 5%. Cultivation was carried out in flasks on a shaker (220 rpm) at 30°C for 16–40 h.

The determination of the oxidation rates of substrates by intact *Acinetobacter* sp. 1NG cells. The oxidation rates of substrates were determined from the respiration rates of intact *Acinetobacter* sp. 1NG cells incubated in a 50 mM Tris–phosphate buffer (pH 7.0) in the presence of one of the substrates (ethanol, acetaldehyde, acetate, and the TCA cycle intermediates). The respiration rates were measured using a PPT-1 polarograph equipped with a Clark-type oxygen electrode [2, 3]. The concentration of the respiration substrates, which were added to the medium in the form of

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potassium salts, was 10 mM. Cells for polarographic studies were grown in the liquid medium to the exponential phase (16–20 h of growth), harvested by centrifugation at 4000 *g* for 15 min (4°C), and washed twice with a 50 mM K-phosphate buffer (pH 7.0).

Enzyme assay. Cell-free extracts for enzyme assay were prepared from cells washed with a 0.05 M K-phosphate buffer (pH 7.0) [2, 3].

The activities of the key enzymes of C_2-metabolism, namely, NAD^+-linked alcohol dehydrogenase (EC 1.1.1.1), $NADP^+$-linked acetaldehyde dehydrogenase (EC 1.2.1.4), acetyl-CoA synthetase (EC 6.2.1.1), acetate kinase (EC 2.7.2.1), and isocitrate lyase (EC 4.1.3.1), as well as 2-oxoglutarate dehydrogenase (EC 1.2.4.2) and pyruvate carboxylase (EC 6.4.1.1), were measured as described elsewhere [2, 3].

Malate synthase (EC 4.1.3.2) was assayed from the decline in the concentration of glyoxylate phenylhydrazone measured colorimetrically at 324 nm [4].

The activities of the NAD^+-linked isocitrate dehydrogenase (EC 1.1.1.41), malate dehydrogenase (EC 1.1.1.37), and glutamate dehydrogenase (EC 1.4.1.2) were determined from the rate of NAD^+ reduction [5–7]. The activities of the $NADP^+$-linked isocitrate dehydrogenase (EC 1.1.1.42) and malate dehydrogenase (EC 1.1.1.82) were determined from the rate of $NADP^+$ reduction [8, 9]. The reduction of NAD^+ and $NADP^+$ was measured spectrophotometrically at 340 nm.

Citrate synthase (EC 4.1.3.7) was assayed from the decline in the concentration of acetylphosphate in the presence of coenzyme A and oxaloacetate [8]. The activities of aconitate hydratase (EC 4.2.1.3), PEP carboxykinase (EC 4.1.1.49), PEP synthase (EC 2.7.9.1), and oxaloacetate decarboxylase (EC 4.1.1.3) were determined from the rate of NADH oxidation measured spectrophotometrically at 340 nm [10–12]. The activity of glutamate dehydrogenase (EC 1.4.1.4) was assayed from the rate of NADPH oxidation measured at 340 nm [13]. Alternatively, PEP synthase was assayed from the decline in the concentration of pyruvate, which was analyzed colorimetrically at 445 nm by reaction with dinitrophenylhydrazine [12].

The NAD^+- and $NADP^+$-linked oxaloacetate-decarboxylating malate dehydrogenases (EC 1.1.1.38 and EC 1.1.1.40, respectively) were assayed by the reduction of NAD^+ and $NADP^+$, respectively [3].

The activity of succinate dehydrogenase (EC 1.3.99.1) was determined from the rate of dichlorophenolindophenol reduction (measured at 600 nm) in the presence of phenazine methosulfate [10]. Fumarate hydratase (EC 4.2.1.2) was assayed from the rate of fumarate formation or consumption measured spectrophotometrically at 315 nm [10].

The activities of all enzymes were measured at a temperature of 28–30°C, which is optimal for the growth of *Acinetobacter* sp. 1NG, and expressed in nmol/(min mg protein). Protein was quantified by the Bradford method [14].

Table 1. The respiration rates of intact *Acinetobacter* sp. 1NG cells incubated in the presence of different C_2-compounds and the TCA cycle intermediates

Respiration substrate	The respiration rate (in nmol O_2/(min mg cells)) of cells grown on		
	ethanol	ethanol + fumarate	ethanol + fumarate*
Ethanol	155.3	149.3	153.2
Acetaldehyde	149.6	152.4	146.9
Acetate	138.7	143.3	103.2
Isocitrate	129.5	180.6	172.3
2-Oxoglutarate	106.3	102.3	98.5
Succinate	141.5	150.3	148.5
Fumarate	77.5	162.4	158.3
Malate	116.8	171.4	167.1

* In this case, pantothenic acid was absent from the growth medium.

RESULTS AND DISCUSSION

The measurement of the respiration of the ethanol-grown *Acinetobacter* sp. 1NG cells oxidizing various intermediates of the TCA cycle showed that these cells are able to oxidize all of the organic acids studied at a rate of 100–140 nmol O_2/(min mg cells), except for fumarate, which was oxidized at a lower rate, 77.5 nmol O_2/(min mg cells) (Table 1). These data suggest that the *Acinetobacter* sp. 1NG cells grown on ethanol contain the complete TCA cycle. This suggestion was confirmed by the results of enzymatic measurements presented in Table 2.

When microorganisms are cultivated on carbohydrate substrates, the TCA cycle serves as the major source of NADH, whose oxidation in aerobic heterotrophs is coupled to energy conservation in the form of ATP. In addition, the TCA cycle produces some intermediates necessary for constructive metabolism [15]. In the *Acinetobacter* sp. cells grown on ethanol, the first energy-yielding reactions of ethanol metabolism are catalyzed by NAD^+-linked dehydrogenases. The cellular pool of C_4-dicarboxylic acids is replenished with the aid of the glyoxylate cycle, whose key enzymes are isocitrate lyase and malate synthase. Malate is oxidized to oxaloacetate by NAD^+- and $NADP^+$-linked enzymes (Table 2). Thus, the complete TCA cycle (i.e., containing active 2-oxoglutarate dehydrogenase) is dispensable to this organism. Nevertheless, the cell-free extract of *Acinetobacter* sp. 1NG was found to contain all

Table 2. The activity of the central metabolism enzymes in the *Acinetobacter* sp. 1NG cells grown under different conditions

Enzyme	Enzymatic activity (in nmol/(min mg protein)) in cells grown on		
	ethanol	ethanol + fumarate	ethanol + fumarate*
NAD^+-linked alcohol dehydrogenase	315.3	342.5	330.6
$NADP^+$-linked acetaldehyde dehydrogenase	234.7	259.0	246.8
Acetyl-CoA synthetase	213.5	ND	ND
Acetate kinase	21.6	ND	ND
Isocitrate lyase	312.2	459.3	17.2
Malate synthase	141.2	215.6	91.2
Citrate synthase	405.6	ND	ND
Aconitase	349.0	ND	ND
NAD^+-linked isocitrate dehydrogenase	0	0	0
$NADP^+$-linked isocitrate dehydrogenase	717.8	1289.6	1739.1
2-Oxoglutarate dehydrogenase	93.4	ND	ND
Succinate dehydrogenase	197.5	ND	ND
Fumarase	186.8	351.3	218.6
NAD^+-linked malate dehydrogenase	643.1	1286.2	1484.1
$NADP^+$-linked malate dehydrogenase	79.8	156.8	113.5
NAD^+-linked glutamate dehydrogenase	642.6	ND	ND
$NADP^+$-linked glutamate dehydrogenase	257.2	ND	ND
NAD^+-linked malate dehydrogenase (decarboxylating)	17.9	ND	ND
$NADP^+$-linked malate dehydrogenase (decarboxylating)	0	ND	ND
Oxaloacetate decarboxylase	446.6	ND	ND
Pyruvate carboxylase	160.6	271.8	192.9
Phosphoenolpyruvate carboxykinase	48.7	362.3	171.5
Phosphoenolpyruvate synthase	487.0	728.5	702.0

Notes: Enzymatic activities were measured in the cell-free extract of mid-exponential cells grown for 16–18 h. ND stands for "not determined."
* In this case, pantothenic acid was absent from the growth medium.

enzymes of the TCA cycle, including, albeit at a lower level, 2-oxoglutarate dehydrogenase (Table 2).

Noteworthy is the high activity of the $NADP^+$-linked isocitrate dehydrogenase in this bacterium. It is known that the flow of isocitrate through the bacterial glyoxylate cycle is regulated by means of the covalent modification (reversible phosphorylation–dephosphorylation) of isocitrate dehydrogenase with the aid of bifunctional kinase/phosphatase [16]. When enterobacteria grow on C_2-substrates, isocitrate dehydrogenase is phosphorylated and its activity drops, whereas the activity of isocitrate lyase rises [16]. The high activity of both isocitrate dehydrogenase and isocitrate lyase in the ethanol-grown *Acinetobacter* sp. suggests that the TCA cycle of this bacterium serves predominantly biosynthetic functions, as was shown earlier for some methylotrophic bacteria [15].

To elucidate the role of the TCA cycle in the C_2-metabolism of *Acinetobacter* sp., we measured the activity of glutamate dehydrogenase, the enzyme that catalyzes the reductive amination of 2-oxoglutarate and the synthesis of amino acids of the glutamate family. The cell-free extract of *Acinetobacter* sp. cells contained both $NADP^+$- and NAD^+-linked glutamate dehydrogenases (Table 2). The activity of these enzymes measured in the direction of glutamate synthesis was rather high, although the desaminating activity of glutamate dehydrogenase was close to zero. These data confirm the suggestion that the TCA cycle in the ethanol-grown *Acinetobacter* sp. cells serves biosynthetic functions.

When microorganisms grow on two- or three-carbon substrates (as pyruvate, lactate, ethanol, and acetate) or substrates (as higher *n*-alkanes, fatty acids, and aromatic compounds) whose catabolism yields acetyl-CoA or the TCA cycle intermediates, they require carbohydrate molecules for the synthesis of nucleic acids, polysaccharides, and some metabolites. The necessary carbohydrates are synthesized in gluconeogenesis [15].

The ability of *Acinetobacter* sp. to grow on C_2- and C_3-substrates and to synthesize EPS [1] suggests that the activity of gluconeogenesis in this bacterium is high. In most microorganisms, the key enzyme of gluconeogenesis is PEP carboxykinase, which is known to catalyze the ATP-dependent conversion of oxaloacetate to PEP [15]. The activity of this enzyme in the cell-free extract of *Acinetobacter* sp. was relatively low (40–50 nmol/(min mg protein) (Table 2) in the presence of any of the three nucleotide triphosphates, ATP, GTP, and ITP.

In general, pyruvate kinase cannot convert pyruvate into PEP, since the equilibrium of the reaction catalyzed by this enzyme is shifted toward the hydrolysis of PEP. However, in bacteria, PEP can be produced in the reaction catalyzed by PEP synthase and linked with the hydrolysis of ATP to AMP [17, 18]. This reaction is thermodynamically feasible, since the hydrolysis of ATP to AMP yields more energy than does the hydrolysis of ATP to ADP.

The cell-free extract of the ethanol-grown *Acinetobacter* sp. cells contained both key enzymes of gluconeogenesis, PEP carboxykinase, and PEP synthase (Tables 2, 3). The activity of PEP carboxykinase in the stationary growth phase was about tenfold higher than it was in the exponential growth phase, whereas the activity of PEP synthase in the mid-exponential and the early stationary growth phases was much the same.

In *Escherichia coli* cells grown on pyruvate, PEP synthase catalyzes the conversion of pyruvate to PEP, whereas PEP carboxykinase is active in the succinate-grown cells [18]. Of interest is the fact that active PEP synthase was detected not only in the *E. coli* cells grown on the substrates involved in gluconeogenesis, but also in the glucose-grown cells [17]. The presence of active PEP synthase in the *E. coli* cells led to the operation of a futile cycle involving PEP and pyruvate. The increase in the PEP synthase activity enhances the PEP/pyruvate ratio and thereby activates the operation of the PEP–phosphotransferase system and stimulates the consumption and catabolism of glucose [17].

In *Acinetobacter* sp. cells grown on C_2-substrates, pyruvate was formed from oxaloacetate in the reaction catalyzed by oxaloacetate decarboxylase (Table 2). Pyruvate is a component of the *Acinetobacter* sp. exopolysaccharide ethapolan [1] and the precursor of amino acids of the pyruvate family [15, 19]. In the cell-free extract of the ethanol-grown *Acinetobacter* sp. cells, the $NADP^+$-linked oxaloacetate decarboxylating malate dehydrogenase (malic enzyme) was absent, whereas the activity of the NAD^+-linked malic enzyme was low, 15–20 nmol/(min mg protein). At the same time, the activity of pyruvate carboxylase was relatively high. This enzyme catalyzes the carboxylation of pyruvate into oxaloacetate [19] and is likely able to catalyze the reverse reaction of oxaloacetate decarboxylation [20]. In the ethanol-grown *Acinetobacter* sp. cells, pyruvate carboxylase, together with oxaloacetate decarboxylase, may be involved in the formation of pyruvate. It should be noted in this regard that pyruvate carboxylase activity in the stationary-phase *Acinetobacter* sp. cells was more than threefold higher than it was in the exponential-phase cells and reached almost 589.4 nmol/(min mg protein). Nevertheless, the physiological role of pyruvate kinase in *Acinetobacter* sp. remains unclear and requires further study.

Table 3. The activity of enzymes involved in gluconeogenesis in the *Acinetobacter* sp. 1NG cells grown on ethanol to different growth phases

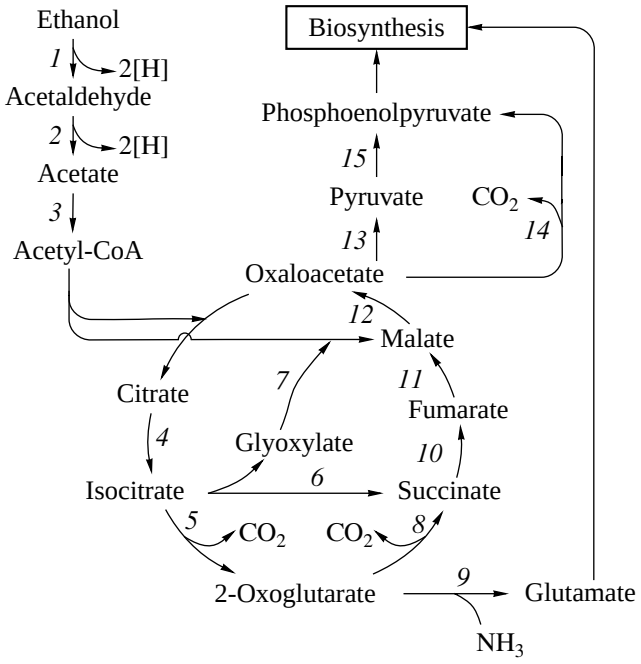
Growth phase	Activity, nmol/(min mg protein)	
	PEP carboxykinase	PEP synthase
Mid-exponential phase	48.7	487.0
Early stationary phase	534.0	508.1

The results of the enzymatic studies described here can be summarized in the following putative scheme of ethanol metabolism in *Acinetobacter* sp. (see figure).

Our earlier studies [1] showed that the synthesis of EPS by *Acinetobacter* sp. can be enhanced by adding C_4-dicarboxylic acids (the intermediates of ethanol metabolism involved in gluconeogenesis) to the growth medium. In particular, the addition of fumarate in 0.2% batches to the stationary-phase *Acinetobacter* sp. culture grown in the ethanol-containing medium augmented the concentration of EPS to 10–15 g/l (by four to five times) and increased the yield of EPS with respect to the substrate consumed (ethanol + fumarate) to 80%. In the light of this, of interest was to study the effect of fumarate on the activity of enzymes involved in gluconeogenesis.

Experiments showed that the addition of fumarate to the medium containing ethanol and pantothenic acid increased the activity of most of the enzymes studied by 1.5–2 times (Table 2). The increase in the activity was observed not only for enzymes of the glyoxylate cycle, which replenishes the cellular pool of C_4-dicarboxylic acids serving as the gluconeogenesis precursors, but also for the proper enzymes of gluconeogenesis. The increase in the activity of PEP carboxykinase in response to the addition of fumarate was as high as 7.5-fold.

When pantothenic acid was absent from the medium containing ethanol and fumarate, the activities of malate dehydrogenase and isocitrate lyase decreased by 2 and more than 25 times, respectively, whereas the



Ethanol metabolism in *Acinetobacter* sp.: *1*, NAD^+-linked alcohol dehydrogenase; *2*, $NADP^+$-linked acetaldehyde dehydrogenase; *3*, acetyl-CoA synthetase; *4*, aconitase; *5*, $NADP^+$-linked isocitrate dehydrogenase; *6*, isocitrate lyase; *7*, malate synthase; *8*, 2-oxoglutarate dehydrogenase; *9*, NAD^+,$NADP^+$-linked glutamate dehydrogenase; *10*, succinate dehydrogenase; *11*, fumarase; *12*, NAD^+, $NADP^+$-linked malate dehydrogenase; *13*, oxaloacetate decarboxylase; *14*, phosphoenolpyruvate carboxykinase; *15*, phosphoenolpyruvate synthase.

activity of isocitrate dehydrogenase somewhat increased (Table 2). In this case, the activities of PEP carboxykinase and PEP synthase (the enzymes of gluconeogenesis) were higher than in the *Acinetobacter* sp. cells grown in the medium with ethanol and pantothenate but without fumarate (Table 2).

The addition of fumarate to the growth medium also enhanced the oxidation of fumarate, malate, and isocitrate by intact *Acinetobacter* sp. cells (Table 1). In the presence of pantothenate, the addition of fumarate to the ethanol-containing growth medium did not influence the oxidation of ethanol, acetaldehyde, and acetate by *Acinetobacter* sp. cells. In the absence of pantothenate, the addition of fumarate somewhat decreased the rate of acetate oxidation by bacterial cells (Table 1).

Thus, experiments showed that the ethanol-grown *Acinetobacter* sp. cells contain both the glyoxylate cycle and the complete TCA cycle. Carbohydrates are formed in the gluconeogenetic processes. The formation of pyruvate and glutamate is catalyzed by oxaloacetate decarboxylase and glutamate dehydrogenase, respectively. The cultivation of *Acinetobacter* sp. in the medium containing ethanol and fumarate leads to an increase in the activity of enzymes of the glyoxylate cycle and major biosynthetic pathways. The data obtained can be used to devise a new biotechnology for the controlled synthesis of ethapolan with the required physicochemical properties.

REFERENCES

1. Grinberg, T.A., Pirog, T.P., Malashenko, Yu.R., and Pinchuk, G.E., *Mikrobnyi sintez ekzopolisakharidov na C_1- i C_2-soedineniyakh* (Microbial Synthesis of Exopolysaccharides from C_1- and C_2-Compounds), Kiev: Naukova Dumka, 1992.
2. Pirog, T.P., Sokolov, I.G., Kuz'minskaya, Yu.V., and Malashenko, Yu.R., Peculiarities of Ethanol Metabolism in an *Acinetobacter* sp. Mutant Strain Defective in Exopolysaccharide Synthesis, *Mikrobiologiya*, 2002, vol. 71, no. 2, pp. 222–229.
3. Pirog, T.P., Kovalenko, M.A., and Kuz'minskaya, Yu.V., Exopolysaccharide Production and Peculiarities of C_6-Metabolism in *Acinetobacter* sp. Grown on Carbohydrate Substrates, *Mikrobiologiya*, 2002, vol. 71, no. 2, pp. 215–221.
4. Pearce, J. and Carr, N.G., The Metabolism of Acetate by the Blue–Green Algae *Anabaena variabilis* and *Anacystis nidulans*, *J. Gen. Microbiol.*, 1967, vol. 49, no. 2, pp. 301–313.
5. Kornberg, A., Isocitric Dehydrogenase of Yeast (DPN), *Methods Enzymol.*, 1955, vol. 1, pp. 707–709.
6. Kitto, G.B., Intra- and Extramitochondrial Malate Dehydrogenase from Chicken and Tuna Heart, *Methods Enzymol.*, 1969, vol. 13, pp. 106–116.
7. Strecker, H.J., L-Glutamic Dehydrogenase from Liver, *Methods Enzymol.*, 1955, vol. 5, pp. 220–225.
8. O'Brien, R.W. and Stern, J.R., Requirement for Sodium in the Anaerobic Growth of *Aerobacter aerogenes* on Citrate, *J. Bacteriol.*, 1969, vol. 98, no. 2, pp. 388–393.
9. Johnson, H.S. and Hatch, M.D., Properties and Regulation of Leaf Nicotinamide Adenine Dinucleotide Phosphate–linked Malate Dehydrogenase and "Malic" Enzyme in Plants with the C_4-Dicarboxylic Acid Pathway of Photosynthesis, *Biochem. J.*, 1970, vol. 119, no. 2, pp. 273–280.
10. O'Brien, R.W. and Geisler, J., Citrate Metabolism in *Aerobacter cloacae*, *J. Bacteriol.*, 1974, vol. 119, no. 3, pp. 661–665.
11. Huei-Che Chang and Lane, M.D. The Enzymatic Carboxylation of Phosphoenolpyruvate: II. Purification and Properties of Liver Mitochondrial Phosphoenolpyruvate Carboxykinase *J. Biol. Chem.*, 1966, vol. 241, no. 10, pp. 2413–2420.
12. Cooper, R.A. and Kornberg, H.L., Phosphoenolpyruvate Synthetase, *Methods Enzymol.*, 1969, vol. 13, pp. 309–314.
13. Martinez-Bilbao, M., Martinez, A., Urkijo, I., Llama, M.J., and Serra, J.L., Induction, Isolation, and Some Properties of the NADPH-Dependent Glutamate Dehydrogenase from the Nonheterocystous Cyanobacterium *Phor-*

midium laminosum, *J. Bacteriol.*, 1988, vol. 170, no. 10, pp. 4897–4902.

14. Bradford, M., A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein–Dye Binding, *Anal. Biochem.*, 1976, vol. 72, pp. 248–254.

15. Malashenko, Yu.R., Sokolov, I.G., and Romanovskaya, V.A., *Mikrobnyi metabolizm nerostovykh substratov* (Microbial Metabolism of Nongrowth Substrates), Kiev: Naukova Dumka, 1987.

16. Cozzone, A.J., Regulation of Acetate Metabolism by Protein Phosphorylation in Enteric Bacteria, *Annu. Rev. Microbiol.*, 1998, vol. 52, pp. 127–164.

17. Patnaik, R., Roof, W.D., Young, R.F., and Liao, J.C., Stimulation of Glucose Catabolism in *Escherichia coli* by a Potential Futile Cycle, *J. Bacteriol.*, 1992, vol. 174, no. 23, pp. 7527–7532.

18. Chao, Y.P., Patnaik, R., Roof, W.D., Young, R.F., and Liao, J.C., Control of Gluconeogenic Growth by *pps* and *pck* in *Escherichia coli*, *J. Bacteriol.*, 1993, vol. 175, no. 21, pp. 6939–6944.

19. Jitrapakdee, S. and Wallace, J.C., Structure, Function, and Regulation of Pyruvate Carboxylase, *Biochem. J.*, 1999, vol. 340, pp. 1–16.

20. Attwood, P.V. and Cleland, W.W., Decarboxylation of Oxaloacetate by Pyruvate Carboxylase, *Biochemistry*, 1986, vol. 25, no. 25, pp. 8191–8196.