ISSN 0026-2617, Microbiology, 2008, Vol. 77, No. 3, pp. 261–267. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © S.M. Trutko, V.A. Shcherbakova, I.V. Ivanova, V.Ya. Lysanskaya, O.V. Arkhipova, N.A. Chuvil'skaya, B.P. Baskunov, D.N. Ostrovskii, V.K. Akimenko, 2008, published in Mikrobiologiya, 2008, Vol. 77, No. 3, pp. 303–310.

EXPERIMENTAL ARTICLES

Interrelation between the Pathways of Isoprenoid Biosynthesis and Carbon Source Catabolism in Anaerobic and Facultatively Anaerobic Bacteria

S. M. Trutko[†a,1], V. A. Shcherbakova[a], I. V. Ivanova[a], V. Ya. Lysanskaya[a], O. V. Arkhipova[a], N. A. Chuvil'skaya[a], B. P. Baskunov[a], D. N. Ostrovskii[b], and V. K. Akimenko[a]

[a] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, 142290 Russia*

[b] *Bach Institute of Biochemistry, Russian Academy of Sciences, Leninskii pr. 33, Moscow, 117071 Russia*

Received February 6, 2007; in final form, May 7, 2007

Abstract—Data on the interrelation between the pathways of the carbon source catabolism and isoprenoid biosynthesis in anaerobic and facultatively anaerobic bacteria were obtained. Two pathways of isoprenoid biosynthesis (nonmevalonate and mevalonate) were revealed in the representatives of the genus *Clostridium.* The nonmevalonate pathway of isoprenoid biosynthesis and the glycolytic pathway of substrate oxidation are typical of glucose-grown bacteria, whereas the pentose phosphate cycle operates in xylose-grown bacteria. The mevalonate pathway of isoprenoid biosynthesis was revealed in strain *Clostridium thermosaccharolyticum* DSM 571 grown in the presence of mevinolin, as well as in a number of lactic acid bacteria. Mevinolin is known to react with the lactate dehydrogenase complex, preventing reduction of pyruvate. The nonmevalonate pathway of isoprenoid biosynthesis was revealed in *Bifidobacterium bifidum.* The role of different metabolic pathways in isoprenoid biosynthesis is discussed.

Key words: Clostridium spp., *Carnobacterium* spp., lactic acid bacteria, mevinolin, fosmidomycin, ^{13}C-glucose, isoprenoid biosynthesis, catabolism of substrates.

DOI: 10.1134/S0026261708030028

Both in higher animals and prokaryotes, isoprenoid synthesis occurs via successive condensation of isopentenyl diphosphate monomers [1], which, in turn, are formed by the condensation either of three acetyl-CoA molecules (the mevalonate pathway) or of pyruvate and 3-phosphoglyceric aldehyde (the nonmevalonate pathway). The nonmevalonate pathway is widespread among eubacteria [1]; it is typical of pathogenic bacteria causing tuberculosis, plague, typhus, anthrax, and cholera. This pathway is inhibited by fosmidomycin and active oxygen.

Earlier, we tested a number of prokaryotes grown in media containing glucose as the source of carbon and energy; glucose metabolism was accompanied by formation of pyruvate and/or 3-phosphoglyceric aldehyde (PGA) [2]. The latter is not only a basic substrate for isoprenoid synthesis, but also plays the role of inductor of the GlpT transport system, which is able to transfer fosmidomycin into the cells [1]. Many microorganisms, along with glucose, utilize pentoses, alcohols, acids, hydrocarbons, etc. as carbon sources. The question arises of whether the adaptation of prokaryotes to utilization of a new substrate is associated with an alteration in the pathway of isoprenoid biosynthesis, which is one of the main anaplerotic processes. The answer to this question is not as obvious as it appears at first sight. There is no way to predict the pathway of isoprenoid biosynthesis in aerobic prokaryotes under replacement of one sugar by another (e.g., hexose by pentose). The reaction of anaerobic microorganisms exhibiting only the substrate phosphorylation route is more predictable.

The limiting stage of the nonmevalonate pathway is known to be the functioning of the first enzyme, 1-deoxy-D-xylulose-5-phosphate synthase [1]. This enzyme has high values of the Michaelis constant for both substrates (pyruvate and PGA). The induction of this synthase depends on the intracellular concentrations of pyruvate and PGA, which, in turn, depend on the carbon source metabolism. Since the C_3-intermediates are formed from hexoses in larger amounts than from pentoses, the former carbon sources are more favorable for the functioning of the nonmevalonate pathway. When anaerobic bacteria are cultivated in the media with pyruvate, lactate, or acetate, the concentrations of substrates for the synthase can decrease signif-

[†] Deceased.

[1] Corresponding author; e-mail: shcherb@ibpm.pushchino.ru

icantly; in this case, activity of the key enzyme must fall to zero and the isoprenoid synthesis will proceed via the three enzymes of the mevalonate pathway [1]. These enzymes can provide for the synthesis of not only the structural elements of cells, but also a number of fermentation products (organic acids and alcohols) that cause the deletion of proton excess, the obligatory process for living organisms. Thus, the dependence of the pathway of isoprenoid biosynthesis on the carbon source in the medium seems to be quite probable.

The goal of the present work was to verify the assumption of the occurrence of an interrelation between the pathways of carbon source catabolism and of isoprenoid biosynthesis in anaerobic and facultatively anaerobic bacteria.

MATERIALS AND METHODS

Cultures. The study was carried out with bacterial strains *Clostridium thermosaccharolyticum* DSM 571, *Thermoanaerobacter lactoethylicum* ZE-1 MGU, *Clostridium* sp. 24 VKM B-2200, *Clostridium* sp. 14 VKM B-2201, *Cl. algoriphilum* VKM B-2271, *Cl. thermocellum* F7 VKM B-2203, *Cl. bowmanii* DSM 14206, *Cl. psychrophilum* DSM 14207, *Carnobacterium funditum* DSM 5972, and *C. alterfunditum* DSM 5970 kept at the Laboratory of Anaerobic Metabolism of Microorganisms, Institute of Biochemistry and Physiology of Microorganisms, Russ. Acad. Sci. Strains *Bifidobacterium bifidum* VKPM AC-1248, *B. adolescentis* VKPM AC-1245, and *B. longum* VKPM AC-1250 were obtained from the All-Russian Collection of Industrial Microorganisms. Strain *Lactobacillus buchneri* VKM B-1599 was obtained from the All-Russian Collection of Microorganisms.

Cultivation conditions. Bacteria of the genera *Thermoanaerobacter* and *Clostridium* were grown in the PYG medium; *Carnobacterium*, in medium 141 [5]. Glucose, gluconate, or xylose served as the carbon sources. Bifidobacteria were grown in medium containing the following (g/l): casein hydrolysate, 15.0; yeast extract, 5.0; glucose, 5.0; cysteine-HCl, 0.5; NaCl, 2.5; $MgSO_4 \cdot 7H_2O$, 0.5; and ascorbic acid, 0.5. Lactobacilli were grown in the medium containing the following (g/l): wort extract, 0.2; yeast extract, 0.2; glucose, 10.0; Tween 80, 1.0; K_2HPO_4 – 2.0; $MgSO_4 \cdot 7H_2O$ – 0.2; $MnSO_4 \cdot 4H_2O$, 0.05; and ammonium citrate, 2.0.

All the cultures studied were grown in Hungate tubes with a medium volume of 10 ml. The inoculum was introduced in an amount of 2–10 vol %, depending on the intensity of the culture growth (2–5 and 10 vol % for cultures with high and relatively low cell density, respectively).

The growth of bacteria was determined from the absorbance at 600 nm with a Spekol 221 spectrophotometer (Germany).

Analytical methods. Fatty acids and alcohols were analyzed on a Pye-Unicam 304 gas chromatograph (United Kingdom) equipped with a flame ionization detector. Fatty acids were determined on a glass column (2 m × 2 mm) packed with 20 wt % neopentyl glycol succinate on Chromosorb W/AW DMCS (100–200 mesh) (Fluka, Germany). A programmed temperature range of 80–175°C was scanned at 6°C/min; injector and detector temperatures were 150 and 180°C, respectively. The flow rate of the CO_2 carrier gas was 20 ml/min. Alcohols were analyzed on a glass column (2m × 2 mm) packed with Porapack QS (80–100 mesh) (Fluka, Germany) in an isocratic regime at temperatures of column, injector, and detector of 100, 120, and 170°C, respectively. Nitrogen carrier gas flow was 20 ml/min [5].

Hydrogen concentration in the gaseous phase was measured on an LKhM-80 gas chromatograph (Russia) equipped with a katharometer and a glass column (1 m × 3 mm) packed with a molecular sieve (30–40 mesh). The temperature of column, injector, and detector was 40°C. Argon carrier gas flow was 20 ml/min [6].

Determination of lactate and pyruvate concentrations was performed by the enzymatic method from absorption intensity of reduced NAD^+ measured spectrophotometrically at 340 nm. In the course of NAD^+ reduction by lactate dehydrogenase, lactate was oxidized to pyruvate; during NADH oxidation by lactate dehydrogenase, pyruvate was reduced to lactate [7].

Determination of glucose and xylose were carried out on a Biotronic 2000 automatic carbohydrate analyzer (Biotronic, Germany) by measuring the intensity of violet coloration of the copper complex with 4,4-2,2-dicarboxy quinoline [8].

Experiments with labeled glucose. Cell cultivation was carried out in bottles containing 200 ml of the medium with ^{13}C-glucose.

Incorporation of ^{13}C-glucose was determined from isotopic composition of CO_2measured on a BreatMath^{plus} Termo Finnigan isotope mass spectometer (Germany) in the automatic sampling mode.

Reagents. Fosmidomycin was obtained from Jomaa Pharmaka GmbH (Germany); cAMP and mevinolin were obtained from Sigma (United States).

RESULTS

The enzymes of sugar degradation in bacteria of the genus *Clostridium* are known to be inducible. In the cells grown on glucose the glycolytic pathway functions, whereas in xylose-grown cells the pentose phosphate cycle operates. In our experiments, the replacement of the six-carbon sugar by the five-carbon substrate resulted in a two- to threefold decrease in biomass yield in most of the bacteria studied (Table 1). The mixture of the inhibitors of isoprenoid biosynthesis (fosmidomycin and mevinolin in a ratio of 1 : 1) inhibited growth of five of the seven strains of clostridia studied by 60 to 80%, whereas two of three strains of

Table 1. The effect of inhibitors of the isoprenoid biosynthesis on the growth of anaerobic bacteria

Culture (growth temperature, °C)	Growth of bacteria (OD_{600}) in the media containing							
	glucose				xylose			
	Control	+F	+M	+F +M	Control	+F	+M	+F +M
Cl. thermosaccharolyticum DSM 571 (55°C)	0.73	0.13	0.85	–	0.37	0.34	0.64	–
	0.64	0.68	0.95	0.16	0.21	0.10	0.41	0.24
	0.67	0.17	1.0	0.24	0.39	0.37	0.83	0.71
Clostridium sp. 24 BKM B-2202 (37°C)	0.57	0.08	0.17	0.17	0.37	0.12	0.50	0.18
Cl. thermocellum (55°C)	0.58	0.11	0.58	0.23	N.d.	N.d.	N.d.	N.d.
T. lactoethylicum ZE-1 (55°C)	1.06	0.99	0.21	0.21	0.12	0.12	0.26	0.18
Cl. algoriphilum BKM B-2271 (5°C)	0.38	0.08	0.23	0.12	0.48	0.06	0.13	0.14
Cl. bowmanii DSM 14206 (12–16°C)	0.59	0.85	0.51	0.48	0.34	0.35	0.24	0.33
Cl. psychrophilum DSM 14207 (5–6°C)	0.48	0.43	0.50	0.38	0.30	0.29	0.27	0.33

Note: +F, +M, and +F +M mean the addition of fosmidomycin (0.1 mg/ml), mevinolin (0.1 mg/ml), and the mixture of inhibitors (0.1 mg/ml each), respectively. N.d. stands for "no data."

psychrophilic bacteria were resistant to these compounds. Fosmidomycin, an inhibitor of the nonmevalonate pathway, suppressed bacterial growth on both glucose- and xylose-containing media; the level of inhibition varied considerably in different experiments. An attempt to increase the effect of fosmidomycin on resistant strains by its addition together with cAMP (1 mM) was unsuccessful. [4]. This finding indicates that alterations in the effect of this inhibitor on bacterial growth were not associated with the lack of adenylate cyclase, a component of the GlpT transport system. Standardization of the inoculum (the use of one-day-old cultures grown on glucose) reduced considerably the scatter of the data (Table 1). Long-term storage of inoculum of *Cl. thermosaccharolyticum* DSM-571 resulted in a loss of the culture sensitivity to fosmidomycin. A similar pattern was observed in *T. lactoethylicum* ZE-1 MGU.

The emergence of cell resistance to fosmidomycin could be due either to the occurrence of a shunt in the nonmevalonate pathway at the level of 1-deoxy-D-xylulose-5-phosphate reductoisomerase [1] or to the function of the second, mevinolin-sensitive pathway of isoprenoid biosynthesis. Mevinolin suppressed growth of *T. lacto-ethylicum* ZE-1 MGY (fosmidomycin-resistant variant), *Clostridium* sp. 24, and the psychrophilic bacterium *Cl. algoriphilum* VKM 2271, but stimulated growth of *Cl. thermosaccharolyticum* DSM 571 (Table 1). In the case of cultivation on xylose, mevinolin also increased the biomass of three of the six strains. The replacement of the carbon source in the medium, e.g., of hexose by pentose, showed no effect on the strain sensitivity to fosmidomycin. The results obtained are indicative of the occurrence of two pathways for isoprenoid biosynthesis in clostridia. The inhibition of the nonmevalonate pathway was associated with a decrease in biomass yield, whereas the inhibition of the mevalonate pathway either decreased or increased the cell density of *Clostridium* sp. 24 grown on glucose or xylose, respectively. The ambiguity of the cell reactions to mevinolin was possibly due to the occurrence in the cells of two inhibitor-sensitive processes, one of which was not involved in biomass synthesis. Assuming that the latter process was fermentation, we compared the composition of the products accumulated in the medium with and without mevinolin. It was revealed that both *Clostridium* sp. 24 and *Cl. thermosaccharolyticum* DSM 571, as well as the other representatives of this systematic group, in the phase of active growth, excreted gaseous hydrogen and acids with different chain lengths (Table 2). In our experiment, fosmidomycin completely inhibited growth of the mesophilic strain *Clostridium* sp. 24, whereas mevinolin showed no effect on the biomass yield on sugar-containing media, but influenced the accumulation of the products, in particular, acetate and hydrogen. Unlike sugar metabolism, the oxidation of gluconate in the presence of mevinolin was not accompanied by a decrease in the hydrogen yield. In the case of the thermophilic strain *Cl. thermosaccharolyticum* DSM 571, mevinolin affected the product accumulation and stimulated cell growth; biomass was maximal on the glucose-containing medium and increased almost twofold in the presence of mevinolin (Table 2).

Table 2. The effect of inhibitors on the product synthesis from various substrates in *Clostridium* sp. 24 and *Cl. thermosaccharolyticum* DSM 571

Cultures Substrate/inhibitor		OD_{600}	Products				
			Acetate, mM	Propionate, mM	Butyrate/isobutyrate, mM	Lactate, mM	H_2, μm
Clostridium sp. 24							
Glucose							
	Control	0.43	5.42	0	2.29/0	4.58	94.0
	Mevinolin	0.37	11.31	0	0.46/0	0.87	61.0
	Fosmidomycin	0	3.2	0	0.23/0	0.62	0.3
	The mixture of inhibitors	0.14	0.68	0	0/0	0.46	0.2
Xylose							
	Control	0.21	4.23	0	1.26/0	0.81	110
	Mevinolin	0.23	2.71	3.01	0.70/0	0.56	68
	Fosmidomycin	0.01	0.68	1.03	0/0	0.71	1.0
	The mixture of inhibitors	0.13	0.68	2.05	0/0	0.66	1.4
Gluconate							
	Control	0.15	2.03	0	0.57/0	0.68	45.0
	Mevinolin	0.08	1.70	0	0.46/0	0.93	42.8
	Fosmidomycin	0.01	0.68	0	0.11/0	0.61	1.1
	The mixture of inhibitors	0.03	0.51	0	0/0	0	0.1
Cl. thermosaccharolyticum							
Glucose	Control	0.64	6.44	0	0/0	2.0	25.2
	Mevinolin	0.95	12.88	0	0/0	1.67	10.8
	Fosmidomycin	0.68	1.86	0	0/0	1.67	25.9
	The mixture of inhibitors	0.16	1.53	0	0/0	2.89	N.d.
Xylose							
	Control	0.21	2.71	0	0/2.41	1.67	27.7
	Mevinolin	0.41	3.38	0	0/3.67	0.89	13.7
	Fosmidomycin	0.10	1.36	0	0/6.43	1.33	25.9
	The mixture of inhibitors	0.24	2.37	0	0/3.33	1.33	N.d.a

Note: N.d. stands for "no data".

The pathway of glucose oxidation in strain *Cl. thermosaccharolyticum* DSM 571 in the presence of mevinolin was determined from the incorporation of ^{13}C-glucose labeled at the C-1, C-2, and C-6 positions. Isotope analysis of evolved CO_2 revealed that only the C-1 atom of glucose was incorporated into the cells (Table 3). This finding indicated that in the presence of mevinolin, the pentose phosphate cycle rather than glycolysis operated in the studied strain.

The stimulatory effect of mevinolin on cell growth was also revealed in other strains (Table 1). In the presence of this inhibitor, xylose expenditure for the biomass synthesis in *Cl. thermosaccharolyticum* DSM 571 decreased two- to threefold (data not shown).

Thus, in the representatives of the genus *Clostridium*, the nonmevalonate pathway of isoprenoid biosynthesis was coupled only with biomass formation, whereas the mevalonate pathway was associated with both biomass production and acid synthesis. Since clostridia are able to involve both pathways of isoprenoid biosynthesis in biomass formation, fosmidomycin can be used as a medical preparation only in the mixture with the other antibiotics, in particular, mevinolin.

The effect of mevinolin on acid production by bacteria was studied. When strain *Clostridium* sp. 24 was grown in the medium with glucose, but not with xylose, it produced equimolar amounts of lactate and acetate (Table 2); mevinolin decreased the concentration of lactate and increased acetate content. This inhibitor also stimulated acetate synthesis by *Cl. thermosaccharolyticum* DSM 571. Therefore, in bacteria of the genus *Clostridium*, mevinolin can regulate overproduction of metabolites at the level of pyruvate.

Mevinolin was shown to decrease the ratio of lactate to acetate in the representatives of the genus *Carnobacterium*, but not in *L. buchneri* (Table 4). Acid production occurred under both anaerobic and aerobic conditions; however, the effect of this inhibitor was observed only in the presence of oxygen. The accumulation of lactic acid in the case of *C. funditum* incubated in the presence of mevinolin (without fosmidomycin) with and without aeration differed by an order of magnitude. This finding is indicative of nonspecific inhibition by mevinolin of reduction of pyruvate to lactate, which resulted in pyruvate accumulation in the medium. The pyruvate concentration reached 60 μM in aerobic culture grown with mevinolin and was absent both in the control variant and in the culture grown in the presence of fosmidomycin. Therefore, mevinolin inhibited lactate dehydrogenase in *C. funditum* under aerobic conditions.

Under anaerobic conditions, both mevinolin and fosmidomycin decreased the growth rate of *L. buchneri* and *C. funditum* by 40 and 60%, respectively. Under aerobic conditions, the inhibitory effect of mevinolin on the growth of *C. funditum* did not exceed 20% and increased to 30% in the presence of the mixture of the two inhibitors. The inhibitors showed no effect on the growth of *C. alterfunditum*; mevinolin affected the production of lactate, but not of acetate. Thus, in *C. funditum*, mevinolin affected two processes: cell growth under anaerobic conditions and lactate accumulation in the presence of oxygen.

Of the three strains of the genus *Bifidobacterium* studied, only *B. longum* was sensitive to mevinolin; growth inhibition reached 90% and the production of acetate and lactate was half that in the control (Table 4). In two other representatives of this genus, mevinolin affected neither acid accumulation nor biomass yield; the effect of fosmidomycin was negligible. The incorporation of C-1-glucose into CO_2 was revealed. Thus, it can be assumed that the strains under study realized different pathways of isoprenoid biosynthesis.

Table 3. Isotope composition of the gaseous phase of the culture *Cl. thermosaccharolyticum* DSM 571

No	Sample	$\delta^{13}C$ ‰	Deviation from initial value
1	Glucose (control)	–25.11	0
2	Glucose + fosmidomycin	–28.70	0
5	Glucose + ^{13}C-2-glucose	–28.86	0
6	Glucose + ^{13}C-2-glucose + mevinolin	–26.69	0
3	Glucose + ^{13}C-1-glucose	–20.62	4.49
4	Glucose + ^{13}C-2-glucose + mevinolin	1.20	26.31

Note: Concentrations of glucose, ^{13}C-glucose, fosmidomycin, and mevinolin were 4.5, 0.1, 0.1, and 0.1 mg/ml, respectively.

DISCUSSION

The results obtained (Tables 1, 2, and 4) demonstrate that the operation of both pathways of isoprenoid biosynthesis in an anaerobic bacterium (*Clostridium*) is the rule rather than an exception. The mixture of inhibitors of two pathways for isoprenoid biosynthesis suppressed cell growth by 60–80%; however, the effect of each of them depended on the cultivation conditions as well as on the culture age.

For example, in glucose-containing medium, the growth of *Cl. thermosaccharolyticum* DSM 571 was inhibited by fosmidomycin but not by mevinolin (Table 3), whereas in the medium with xylose, this bacterium was resistant to both compounds. At the same time, under conditions favorable for glycolysis (Table 1, medium with glucose), the amount of isoprenoids synthesized via the nonmevalonate pathway reached 80%. The contribution of this pathway to cell metabolism decreased in the medium with pentose (xylose). This interpretation is supported by the data shown in Table 1; mevinolin decreased biomass yield of three strains grown in glucose-containing medium, but stimulated growth of the same strains in the medium with xylose. The contradictions between these data can be explained by the assumption that, along with the occurrence of 3-hydroxy-3-methylglutaryl-CoA reductase, there is a second center of antibiotic binding in the cell. In bacteria of the genus *Clostridium*, mevinolin increased acetate concentration and decreased lactate accumulation (Table 2). This finding indicates that the lactate dehydrogenase complex acted as the center of antibiotic binding. This conclusion is supported by the results obtained with the representatives of the genus *Carnobacterium* (Table 4). In the presence of oxygen, mevinolin prevented lactate accumulation, but stimulated production of pyruvate and its oxidation to acetate in *C. funditum.* The latter reaction can serve as a source of ATP for the biosynthesis of cell components that promoted an increase in biomass yield in the presence of this inhibitor (Tables 1 and 2).

At the same time, excretion of lactate by lactic acid bacteria is known to be accompanied by $\Delta\mu H$ formation, which serves as an additional energy source and is involved in the control of the transport of compounds into the cells [9]. That is why the effect of mevinolin on

Table 4. The effect of inhibitors of isoprenoid biosynthesis on the product accumulation by facultatively anaerobic bacteria

Cultures and cultivation conditions	Level of inhibition, %		Products, μM			
			Acetate		Lactate	
	$-O_2$	$+O_2$	$-O_2$	$+O_2$	$-O_2$	$+O_2$
Carnobacterium alterfunditum						
Control			100	89	86	85
Fosmidomycin	0	0	91	84	80	87
Mevinolin	0	0	97	105	83	54
The mixture of inhibitors	0	0	99	106	89	94
L. buchneri						
Control			65	70	87	72
Fosmidomycin	40	0	76	65	63	75
Mevinolin	0	0	66	75	94	83
The mixture of inhibitors	12	0	38	69	97	89
C. funditum						
Control			44	59	56	45
Fosmidomycin	0	8	31	44	56	47
Mevinolin	58	18	37	53	53	5.4
The mixture of inhibitors	44	30	29	56	39	34
B. longum AC-1250						
Control		N.d.	103	N.d.	53	N.d.
Fosmidomycin	+36	N.d.	97	N.d.	43	N.d.
Mevinolin	90	N.d.	45	N.d.	27	N.d.
The mixture of inhibitors	74	N.d.	82	N.d.	36	N.d.
B. adolescentis AC-1245						
Control		N.d.	99	N.d.	52	N.d.
Fosmidomycin	28	N.d.	98	N.d.	63	N.d.
Mevinolin	0	N.d.	68	N.d.	46	N.d.
The mixture of inhibitors	0	N.d.	78	N.d.	41	N.d.
B. bifidum AC-1248						
Control		N.d.	149	N.d.	92	N.d.
Fosmidomycin	20	N.d.	146	N.d.	92	N.d.
Mevinolin	0	N.d.	134	N.d.	75	N.d.
The mixture of inhibitors	0	N.d.	124	N.d.	81	N.d.

Note: N.d. stands for "no data".

the growth of this group of bacteria could also depend on the concentration of excreted lactic acid. In particular, different levels of lactate accumulation in suspensions of *B. longum, C. funditum*, and *C. alterfunditum* can explain the sensitivity of the first strain and resistance of the third one to mevinolin. As for fosmidomycin, its effect on bacteria did not correlate with lactate concentration in the medium (Table 4). For instance, in the case of *L. buchneri*, which produced high concentrations of lactate comparable with those observed in *C. alterfunditum*, fosmidomycin inhibited cell growth only under anaerobic conditions, i.e., under low values of redox potential. Unlike mevinolin, phosphonate suppressed growth of both *B. adolescentis* and *B. bifidum*; the level of growth inhibition was within 30% (Table 4). Incorporation of ^{13}C into the biomass occurred only when glucose was labeled at the C-1 (but not at the C-2) position and increased in the presence of mevinolin. Therefore, the pentose phosphate cycle operated both in *B. bifidum* and *Cl. thermosaccharolyticum* DSM 571 (Table 3). At the same time, the data on the effect of mevinolin on biomass yield of only one of these two

strains are indicative of a difference in the operation of the pathways of isoprenoid biosynthesis in these strains. Further investigations are required to elucidate these distinctions.

It is known that different pathways of the carbon source catabolism can lead to the formation of different concentrations of 3-phosphoglyceric aldehyde (PGA) in the cells. For instance, in the representatives of the genus *Clostridium*, dehydrogenation of glucose occurs at the initial stages of the pentose phosphate pathway; only one molecule of PGA is formed from three molecules of glucose. The sequence of reactions of glucose metabolism in *B. bifidum* was revealed only recently [10]. This fragment of the pentose phosphate cycle proceeded in the reverse direction (from fructose-6-phosphate to sedoheptulose-7-phosphate and then to pentoses and PGA). The oxidation of two molecules of glucose via this pathway resulted in formation of two PGA molecules. Moreover, there are variants of the pathways for isoprenoid biosynthesis involving either acetyl-CoA or PGA; the conversion of the latter involves two ATP molecules and one molecule of NADH. One molecule of acetyl-CoA is formed from one molecule of pyruvate; three molecules of acetyl-CoA are consumed to produce one isoprenoid molecule. Since PGA acts as a "building material" in the nonmevalonate pathway of isoprenoid biosynthesis, the realization of "the reverse pentose phosphate cycle" in bifidobacteria appeared to be the most favorable for isoprenoid synthesis [11]. In other words, the pentose phosphate pathway serves as a point of switching from one metabolic pathway to the other. This pathway seems to play an important ecological role providing for the adaptation of microorganisms to stress conditions.

Thus, the results obtained indicate that the pathway of isoprenoid biosynthesis in anaerobic and facultatively anaerobic bacteria depends on the type of the carbon source metabolism; the amount of PGA formed in the course of the carbon source catabolism plays a key role in the subsequent substrate transformation.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project no. 05-04-49641).

REFERENCES

1. Ershov, Yu.V., Methylerythritol phosphate (Nonmevalonate) pathway of Isoprenoid Biosynthesis, *Usp. Biol. Khim.*, 2005, vol. 45, pp. 307–354.
2. Trutko, S.M., Dorofeeva, L.V., Shcherbakova, V.A., Chuvil'skaya, N.A., Laurinavichus, K.S., Binyukov, V.I., Ostrovskii, D.N., Khintts, M., Visner, I., Iomaa, Kh., and Akimenko, V.K., Occurrence of Nonmevalonate and Mevalonate Pathways for Isoprenoid Biosynthesis in Bacteria of Different Taxonomic Groups, 2005, vol. 74, no. 2, pp. 185–190 [*Microbiology* (Engl. Transl.), vol. 74, no. 2, pp. 153–158].
3. Trutko, S.M., Dorofeeva, L.V., Evtushenko, L.I., Ostrovskii, D.N., Khintts, M., Visner, I., Iomaa, Kh., Baskunov, B.P., and Akimenko, V.K., Isoprenoid Pigments in Representatives of the Family *Microbacteriaceae, Mikrobiologiya*, 2005, vol. 74, no. 3, pp. 335–341 [*Microbiology* (Engl. Transl.), vol. 74, no. 3, pp. 284–289].
4. Trutko, S.M., Dorofeeva, L.V., and Laurinavichyus, K.S., Resistance of Actinobacteria to Fosmidomycin and the Role of cAMP Level, in *2-ya Mezhdunarodnaya konferentsiya "Mikrobnoe raznoobrazie: sostoyanie, strategiya sokhraneniya, biologicheskii potentsial"*, (2nd Int. Conf. "Microbial Diversity: State, Maintenance Strategy, and Biological Potential"), ISOMID-2005, Perm, pp. 100–101.
5. Anonymous, *DSMZ catalogue of strains. 2001, 7th ed.*, Braunschweig, Germany: Deutsche Sammlung von Mikroorganismen und Zellkulturen.
6. Hungate, R.E, A Roll Tube Method for Cultivation of Strict Anaerobes, in *Methods in Microbiology*, Norris, J.B. and Ribbons, D.W., Eds., New York: Academic, 1969, pp. 117–132.
7. Hohorst, H.J, Bestimmung mit Lactat-Dehydrogenase und NAD^+, in *Methods of Enzymatic Analysis*, Bergmeyer, H.Y., Ed., Weinhein: Verlag Chemie, 1970, p. 1425.
8. Sinner, M. and Puls, J., Non-Corrosive Dye Reagent for Detection of Reducing Sugars in Borate Complex Ion-Exchange Chromatography, *J.Chrjmatography*, 1978, vol. 156, pp. 197–204.
9. Skulachev, V.P., *Energetika biologicheskikh membran* (Energetics of Biological Membranes), Moscow: Nauka, 1989.
10. *Biology of the Prokaryotes*, Lengeler, J.W., Drevs, G., and Shlegel, G., Eds., Stuttgart: Georg Thieme, 1999 [Russ. Transl. *Sovremennaya mikrobiologiya. Prokarioty*, Moscow: Mir, 2005, vol. 1, p. 654].
11. Ershov, Yu.V., Gantt, R.R., Cunningham, F.X., and Gantt, E., Isoprenoid Biosynthesis in *Synechocystis* sp. Strain PCC6803 Is Stimulated by Compounds of the Pentose Phosphate Cycle but Not by Puruvate or Deoxyxylulose-5-Phosphate, *J. Bacteriol.*, 2002, vol. 184, no. 18, pp. 5045–5051.